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THE SUSCEPTIBILITY AND IMMUNITY OF CERTAIN MARINE FISHES TO *EPIBELLA MELLENI*, A MONOGENETIC TREMATODE

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INTRODUCTION

In recent years the development of resistance to metazoan parasites has been demonstrated in several host species. Investigations of Ackert, Africa, Cort, McCoy, Miller, Stoll, and others have shown definitely that an immunity is developed against such helminthic parasites as cestodes and nematodes. Much experimental evidence has been obtained on the nature of this resistance and the results of these investigations were summarized by Chandler (1932). He states that the "Resistance to injurious effects of infection is influenced by species and race of host, diet, age, intercurrent disease, and other factors which lower the reserve vitality of the host. Resistance to acquisition and continued accumulation of worms is influenced by species of host, age, diet, vitamin deficiency, and existing and pre-existing infection with the same species of worm. The development of acquired resistance is shown not only by failure of the hosts to acquire more parasites, but also by the loss of parasites already established, by partial inhibition of reproduction, by retardation in rate of growth and decrease in size of worms, and in some cases by complete inhibition of development. Evidence is afforded that the immunity is at least in part local in nature, that blood changes play little if any part, and that the acquired resistance is highly specific in its nature."

Up to the present very little is known about immunity against trematode infections. In so far as the writers were able to determine, there are only two definite records. Fujinami (1916) and Fujinami and Sueyasu (1917) reported that a horse and some calves became immune to the blood fluke, *Schistosoma japonicum*, after an infection of short duration. The other case was reported by Donham, Simms and Miller (1926). These investigators showed that infection in dogs with the intestinal fluke, *Nanophyetus salmincola*, produced the so-called "salmon poisoning." In most cases the infection proved fatal, but in a few instances the dogs that survived afterwards acquired a resistance to the parasites.

Likewise information concerning acquired resistance to external metazoan parasites is just as scanty. Reuling (1919) found that the large mouth black bass, *Micropterus salmoides*, acquired a resistance after two or three heavy infections with the glochidia of the fresh-water mussel, *Lampsilus luteola*. It was found that the glochidia would tend to slough off shortly after encysting on the fish, and by *in vitro* experiments Reuling showed definitely that the fish serum acquired a lytic property which was capable of destroying the glochidia.

Blacklock and Thompson (1923) and Blacklock and Gordon (1927) have shown that an immunity was developed by man, dog, monkey, and guinea-pig to the larvae of the insect, *Cordylobia anthropaga*. They came to the conclusion that the general circulation and the deeper tissues took no part in the destruction of the larvae. The immunity is confined to areas of the skin previously inoculated with the larvae. This acquired resistance spreads from the primary focus, so that areas which have never been the site of invasion by the parasites may ultimately become immune. According to the above authors, the resistance lasted for at least three months.

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OBSERVATIONS ON IMMUNITY OF MARINE FISHES TO *Epibdella*

With the exception of the work of Jahn and Kuhn (1932), we have found no records of *acquired resistance to external trematode parasites*. These investigators, in their study of the life-history of *Epibdella meleni*, a monogenetic trematode from the eyes and skin of marine fishes, reported that a large number of these animals were found to be susceptible to infection. Among the species listed by them, the following developed a partial immunity (immunity to super-infection) after a period of susceptibility: *Caranx crysos*, runner, *Caranx hippos*, common jack, *Centropristes striatus*, common sea-bass, *Dermatolepis punctatus*, spotted grouper, *Epinephelus morio*, red grouper, *Epinephelus striatus*, Nassau grouper, *Lutianus synagris*, spot snapper, *Melichthys bispinosus*, black triggerfish, and *Spheroides annulatus*, Pacific puffer. As stated by these investigators, "Most of these species are usually present in the tanks of the Aquarium, and newly arrived specimens always show a marked susceptibility to infection. However, after being present in the tanks for several weeks these species seldom show a slight and never a serious infection, although they are continually exposed to re-infection."

It is interesting to point out a fact that has come to the attention of the writers, that with the addition of newly arrived fishes, the parasites always show a tremendous increase in numbers. With the latest additions of Bermuda fishes (June and September, 1932) to the waters

of the New York Aquarium, a new but less severe outbreak has occurred. From June, 1931, until the present time, repeated observations were made to determine whether any of the above mentioned species would develop a *total immunity*, and, whether or not other species, not previously mentioned, would become susceptible to infection or develop such a resistance. If any of the previously infected fishes had acquired an immunity, it is believed that this latest epidemic would demonstrate that fact.

As pointed out by Taliaferro (1929) "Although such terms as immunity and host's resistance are extremely useful, they are always, . . . relative. Thus, a host may be immune to a parasite, but by subjecting the host to conditions which lower its resistance, or by subjecting the parasite to conditions which enhance its virulence or by disturbing the quantitative relations, as by giving an abnormally large infecting dose of parasites, a seemingly complete immunity may be broken down." Therefore, it must be remembered that infective stages of *E. melleni* are always present in the salt water system of the New York Aquarium and that when additions of new fishes are made, invariably an epidemic occurs. Since all fishes are subjected to the same environment in the aquarium, the conditions mentioned by Taliaferro must occasionally arise and previously immunized fishes may again become susceptible.

Reconsidering the list of susceptible and immune fishes given by Jahn and Kuhn (1932), the writers found that:

(1). *Caranx crysos* (Mitchill), runner,¹ developed a partial immunity. The parasites appear in lesser numbers following a previous heavy infection. (X)²

(2). *Caranx hippos* (Linnaeus), common jack, showed no infection from the time the fish were treated with "sol-argentum."³ A period of over a year.

(3) (4). *Naucrates ductor* (Linnaeus), pilot fish, and *Trachinotus carolinus* (Bloch), common pompano, are always susceptible. Infection invariably light. (X)

(5). *Vomer setapinnis* (Mitchill), moonfish. Of a group that were infected two years ago all died from the infection except one. This individual acquired an immunity that withstood infection during the entire period. This is an interesting case and will be mentioned again.

¹ The arrangement of the fishes in this list is in agreement with their customary systematic position. They belong to the group of spiny-rayed fishes (Order Acanthopteri).

² (X) indicates that the observation is in agreement with that of Jahn and Kuhn.

³ Solutions of "sol-argentum" and similar substances are used in the New York Aquarium for treating infected fishes.

(6). *Pomatomus saltatrix* (Linnaeus), bluefish, are susceptible, but infection almost always light. (X)

(7). *Centropristes striatus* (Linnaeus), common sea-bass. According to Jahn and Kuhn this species developed a partial immunity. Our observations, however, show that they only become parasitized when the trematodes are present in great numbers. At all other times they are entirely free of the worms.

(8). *Dermatolepis punctatus* Gill, Pacific spotted grouper, developed a partial immunity. (X)

(9). *Epinephelus adscensionus* (Osbeck), rock hind. Observations for the past year or more failed to show any parasites on this species, although they were previously infected.

(10). *Epinephelus guttatus* (Linnaeus), red hind. Old individuals of this group, following an attack, have been immune for the past year. During the present epidemic they began to show signs of a slight infection. Newly arrived specimens, however, became severely infected and a number of them have succumbed.

(11) (12). *Epinephelus morio* (Cuvier and Valenciennes), red grouper, and *Epinephelus striatus* (Bloch), Nassau grouper, were entirely free of the parasites for the past year and a half. Jahn and Kuhn reported these as developing a partial immunity. New specimens become heavily infected.

(13). *Paralabrax maculatofasciatus* (Steindachner), Pacific spotted cabrilla. No observations. According to Jahn and Kuhn they are susceptible.

(14). *Promicrops itaiara* (Lichenstein), jewfish. The parasites remain on the fish only during the epidemic. At all other times (comparatively normal periods) the worms attach to the eyes of this species but in a short time they actually drop off.

(15). *Lutianus analis* (Cuvier and Valenciennes), muttonfish. No observations. According to Jahn and Kuhn they are susceptible.

(16). *Lutianus apodus* (Walbaum), schoolmaster. For the past year this species has been immune. Not listed before as developing an immunity.

(17). *Lutianus jocu* (Bloch and Schneider), dog snapper. No parasites were obtained from this species for the past year. Not listed before as developing an immunity.

(18). *Lutianus synagris* (Linnaeus), spot snapper. According to Jahn and Kuhn, developed a partial immunity. Our observations show, however, that this species has been immune since June, 1931.

(19). *Anisotremus surinamensis* (Bloch), black margate, have been immune for the past year.

(20). *Anisotremus virginicus* (Linnaeus), porkfish. This species was entirely free from the parasites for more than a year and during the recent epidemic became heavily infected, resulting in the death of over half of them.

(21) (22). *Haemulon album* Cuvier and Valenciennes, margate and *Menticirrhus saxatilis* (Bloch and Schneider), kingfish, according to Jahn and Kuhn, are susceptible.

(23) (24) (25). *Micropogon undulatus* (Linnaeus), croaker, *Lachnolaimus maximus* (Walbaum), hogfish, and *Tautoga onitis* (Linnaeus), tautog, are always susceptible, but developing only a light infection. (X)

(26) (27) (28). *Chaetodipterus faber* (Broussonet), spadefish, *Angelichthys ciliaris* (Linnaeus), queen angelfish, and *Angelichthys isabelita* Jordan and Ritter, blue angelfish, are always susceptible and very heavily infected. (X)

(29). *Angelichthys townsendi* Nichols and Mowbray, Townsend's angelfish. According to Jahn and Kuhn this species is susceptible, and of the original group observed by them all died except two. These two individuals have not shown any signs of reinfection for the past year and a half.

(30). *Chaetodon ocellatus* Bloch, common butterfly fish, are susceptible but infection light. (X)

(31). *Pomacanthus arcuatus* (Linnaeus), black angelfish, appears to show some sort of age susceptibility. During the epidemic, young specimens showed a very light infection but the parasites soon dropped off. Older members of this species always show a heavy infection.

(32). *Pomacanthus paru* (Bloch), French angelfish,⁴ are very susceptible and the infection is always heavy. (X)

(33) (34). *Acanthurus caeruleus* Bloch and Schneider, blue tang, and *Acanthurus hepatus* (Linnaeus), brown tang or doctor fish, are susceptible but the infection invariably is light. (X)

(35). *Balistes vetula* Linnaeus, queen triggerfish. Although reported as susceptible by Jahn and Kuhn, the writers have observed no infection since June, 1931.

(36). *Melichthys bispinosus* Gilbert, Pacific black triggerfish, appears to have developed a resistance since the investigation of Jahn and Kuhn, and as yet has shown no signs of an infection.

⁴ Species numbered 26, 27, 28, 29, 31 and 32 are the most susceptible fishes in the Aquarium. These were isolated and kept in another circulation in which the density was raised sufficiently to kill the parasites and cause a cessation in the development of the eggs. However, if any of these species are returned to the main circulation, they become readily reinfected. A report on the control of this parasite appeared in the Bulletin of the New York Zoological Society (Nigrelli, 1932).

(37). *Ceratacanthus schoepfi* (Walbaum), orange file fish, are always infected and infection is invariably heavy. (X)

(38). *Stephanolepis hispidus* (Linnaeus), common file fish, appeared immune for the past year and a half. A few, however, recently showed a light infection.

(39) (40) (41). *Lactophrys tricornis* (Linnaeus), cowfish, *L. trigonus* (Linnaeus), common trunkfish, and *L. triqueter* (Linnaeus), smooth trunkfish, according to Jahn and Kuhn are susceptible.

(42). *Spheroides annulatus* (Jenyns), Pacific puffer, was believed by MacCallum (1927) to be the species that introduced the parasite into the Aquarium but for the past year and a half it has been free from any infection. Jahn and Kuhn reported this species as being partially immune.

(43). *Spheroides maculatus* (Bloch and Schneider), common puffer. Although new fish have been added to the tanks, no infestation was observed during the past two years. A large number of puffers were examined for both internal and external parasites but not a single specimen of *Epibdella* was encountered. This species, however, was reported by Jahn and Kuhn as being susceptible.

(44). *Diodon hystrix* Linnaeus, porcupine fish, were reported by Jahn and Kuhn as being susceptible.

(45). *Prinotus evolans* (Linnaeus), striped sea robin. No infection encountered since the beginning of our observations. Susceptible according to Jahn and Kuhn.

(46). *Malacanthus plumeri* (Bloch), sand fish. No observations. Reported as a susceptible fish by Jahn and Kuhn.

The following species known to be susceptible to infection by *Epibdella melleni* are added to the list of Jahn and Kuhn:

(47). *Holocentrus ascensionis* (Osbeck), squirrel fish, will become parasitized during the rage of the epidemics.

(48). *Paranthias furcifer* (Cuvier and Valenciennes), creole fish. A group of this species brought into the Aquarium in June, 1932, became infected and were treated with "sol-argentum." Since then they have been entirely free of the parasites.

(49). *Roccus lineatus* Bloch, striped bass, shows a slight infection when the parasites are present in great numbers.

(50) (51). *Cephalopholis fulvus* (Linnaeus), coney, and *Petrometopon cruentatus* (Lacepède), graysby, are the most susceptible fishes in the Aquarium at the present time. Infection has been so heavy that the disease often proved fatal.

(52) (53). *Lutianus griseus* (Linnaeus), gray snapper, and *Evolplites viridis* (Valenciennes), blue stripe snapper, are susceptible and at times they are heavily infected.

(54) (55). *Haemulon flavolineatum* (Desmarest), yellow grunt, and *Bodianus rufa* (Linnaeus), Spanish hog-fish will become infected during epidemics but in normal periods (when the numbers of parasites have decreased) they recover.

(56). *Chaetodon capistratus* Linnaeus, four-eye fish, has been heavily infected.

(57). *Canthidermis sabaco* (Poey), ocean triggerfish, are always parasitized but infection is invariably light.

Species numbered 49, 52, 53, 54 were considered by Jahn and Kuhn to be non-susceptible. We have found that the species 49 and 54 at normal times do not show an infection. The worms appear only during the height of the epidemic, when the virulence and numbers of the parasites have increased, but always in such small numbers that without careful observations they could be easily missed. Species 50 and 53 have shown an infection at very irregular intervals. During the height of the epidemics, however, the parasites have been continually present.

The species numbered 50 and 51 have not been included in Jahn and Kuhn's list either as a susceptible or non-susceptible fish. As far as could be determined, these two species have always shown an infection and only recently have the parasites appeared so numerous as to become really a serious infection.

In analyzing the data given above, we can separate the results into four categories: (1) those fish that acquire a partial immunity; (2) those which acquire a resistance that withstood infection for more than a year; (3) those which become infected only during the periods of the epidemics; and, (4) those which are always susceptible and show either a light or heavy infection.

DISCUSSION

Thus, from the above list, it can be readily seen that in such a confined space as the Aquarium, a large number of unnatural hosts may become susceptible to infection with *Epibdella melleni*. Which of these species is really the specific host has never been determined. As pointed out by Jahn and Kuhn, MacCallum (1927) believed that the parasites were introduced by a Pacific puffer, *Spherooides annulatus*. This, however, is doubtful in view of the fact that at the time that MacCallum described this parasite from fishes in the New York Aquarium, no Pacific puffers were present. Also, it was definitely known that the parasite was present in Chicago and Philadelphia Aquaria long before they had any Pacific fishes. At the present time there are a few individuals of *S. annulatus* in the New York Aquarium and they have not shown an infection during the entire period of our observations. It is possible, however, that an earlier infection induced an immunity which was able to protect the fish during this period.

There can be no doubt that some sort of resistance is developed, but the degree of immunity produced shows no consistency among the groups studied. The family Serranidae shows all gradations in resistance. Thus, we find that such forms as *Mycteroperca bonaci* (Poey), black grouper, *M. venenosa* (Linnaeus), yellow fin grouper, and *M. microlepis* (Goode and Bean), gag, show a natural immunity and at no time have any of these fishes become infected, although they are continually exposed to the parasites. On the other hand closely related forms, such as the Nassau grouper and the red hind (both members of the genus *Epinephelus*, family Serranidae) at one time showed a very heavy infection, but eventually developed a more or less complete resistance. Newly introduced specimens invariably become heavily infected. In the case of the jewfish (genus *Promicrops*, family Serranidae) the parasites, as was pointed out before, appear on the eyes at the time of the epidemics, but at other periods they are usually uninfected. If they are attacked, the infection is temporary, for the worms soon drop off. As noted by Jahn and Kuhn, . . . "the possibility of the development of immunity seems to be more strongly suggested in this family."

Another example of variation in resistance among the different species of the same family is well illustrated in the case of the file fish, family Monacanthidae. The present epidemic has its focus on the eyes of *Ceratacanthus schoepfi*, orange file fish, yet *Stephanolepis hispidus*, common file fish, very seldom shows an infection, and the specimens that were previously parasitized have been resistant for the past two years.

The most susceptible fishes belong to the family Chaetodontidae. They include *Angelichthys ciliaris*, queen triggerfish, *Angelichthys isabelita*, blue angelfish, *Chaetodon capistratus*, four-eye fish, and *Pomacanthus paru*, French angelfish. The angelfish appear to be foci for epidemics and must be kept isolated from the main circulation. Yet two species, *Angelichthys townsendi* and *Pomacanthus arcuatus*, of this family, show definite signs of resistance although the immunity is of two different types. The former has maintained an immunity for a period of one year and a half. The record is as follows: five specimens present in the tanks in 1930, three died as a result of the infection, two survived and developed an immunity which so far has withstood the present epidemic. In the case of *Pomacanthus arcuatus*, the immunity is apparently correlated with age. The young individuals⁵ become temporarily infected with immature worms, the parasites dropping off in a short time (one to two weeks). The adults, however, always show a heavy infection.

Whether the latter instance is one of age immunity or of natural re-

⁵ Young specimens have a yellow band which disappears when they reach maturity.

sistance is difficult to determine. Age immunity is generally defined as a change in resistance with advance in age, and in most cases the change is toward increased resistance. According to Sandground (1929), "The definition of age resistance stipulates the absence of a previous infection. It is, therefore, unreasonable to suspect that specific antigenic substances, directly antagonistic to the development of the parasite, have developed in the host. . . ." The resistance of immature *Pomacanthus arcuatus* might possibly be explained on the basis of Ehrlich's athreptic immunity. According to this view, the young fish may lack suitable nutritional material for the adult parasites. This seems to be supported by the fact that no adult worms are ever found on young fish.

There are many examples in which resistance to parasites is lowered with an advance in age and among animal parasites this is a well established fact in the sporozoan disease, *Babesia*, where the infection is less severe in young cattle than in adults. On the contrary, Sandground has argued that age immunity is characteristic of abnormal hosts. He suggested that the adults are so specific in certain physiological characters as to be unsuitable for the development of the parasite, while the young are less specifically differentiated and the parasites may gain a temporary foothold.

Another interesting case that came under our observation is that of *Vomer setapinnis*, moonfish. Of an original group of this species brought into the Aquarium two years ago, all except one specimen died as a result of the infection by *E. melleni*. Recently another group of the same species of fish were brought in and placed in the same exhibition tank containing the lone survivor of the last epidemic. In a short time the newly arrived members became heavily parasitized, while the original member remained uninfected. This tank was kept under observation for three weeks following the introduction of the new fish. During the latter part of this period, one of the infected fish died. To prevent the others from the same fate it was decided to treat them with "sol-argentum." The uninfected fish was treated with the group. It is a known fact that moonfish are very delicate and any handling frequently proves injurious. For a week after the treatment no parasites were found on any of the fish, but at the beginning of the second week the original uninfected fish began to show a slight infection while the others remained free. It is the belief of the writers that the original uninfected fish was so weakened in the process of handling that its resistance to the parasite was diminished. However, later observation showed that the other members of this group became reinfected.

The skin of this species of fish is smooth, whitish and glistening; the scales are rudimentary, so that any marks become noticeable. When

they are treated, the scars left by the parasites can be readily seen. When they became reinforced it was noticed that this second infection never occurred in the regions of the previous one. Thus, originally the infection was concentrated around the eyes and just slightly posterior on the dorsal surface. The reinfection took place on the lateral and ventral sides of the body and the parasites never migrated to the area of the first infection. It is possible that this is the type of resistance (skin immunity) that occurs among the fishes in which resistance has been observed. Probably it is of the same kind as that noted by Blacklock and Thompson (1923) and Blacklock and Gordon (1927) for myiasis, except that in the case of the fishes the immunity appears to be more localized.

SUMMARY

1. It has been shown that certain marine fishes in the tanks of the New York Aquarium, after a period of susceptibility to infection with *Epididella melleni*, a monogenetic trematode, will develop an immunity.

2. The degree of immunity produced shows no consistency among the groups studied, varying greatly between members of the same family.

3. In analyzing the data on fishes from which *Epididella* has been taken, it has been found that: (a) some fish acquire a partial immunity; (b) some acquire a total immunity lasting for long periods; (c) certain fishes that appear to have a natural immunity will become slightly infected during periods of epidemics; (d) certain fishes are always susceptible and may show a heavy or light infection.

4. An apparent inverse age immunity appears to be present in the case of *Pomacanthus arcuatus*, black angelfish. In this case the young acquires an immunity after a very short period of susceptibility (one to two weeks). The older members are always parasitized.

5. In the case of *Vomer setapinnis*, moonfish, a skin immunity has been demonstrated. Reinfection never takes place in the region of the previous infection, indicating a tendency toward localized immunity.

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A NEW GENUS AND SPECIES OF PARASITIC TURBEL-
LARIA FROM A BERMUDA SEA CUCUMBER

F. G. MESERVE*

Macrogynium ovalis n. g., n. sp.

External morphology: The parasite is circular to somewhat elliptical in outline, being slightly convex on the dorsal surface and concave on the ventral with a small indentation in the mid-line at the posterior end which is the opening of the genital system. The mouth is almost invisible and consists of an invagination of ectoderm cells on the ventral surface in the mid-ventral line about one-third the distance from the anterior end. The animal is ciliated all over. Specimens killed in Bouin's solution have a maximum width of 554.4 microns and a minimum width of 423.8 microns. Their length varies from 521.6 microns to 782.4 microns. Flattened and stained specimens mounted in toto were somewhat larger due to their margins being extended by pressure. Four mounted specimens vary in width from 716.8 to 994.4 microns and in length from 863.9 to 1141.0 microns. The thickness was determined from sagittal and transverse sections and varies from 146.7 to 309.7 microns. This species, therefore, is a little less than half a millimeter wide, approximately six-tenths of a millimeter in length and two-tenths of a millimeter in thickness.

Digestive system: An oral sucker is absent. The mouth is situated in the mid-ventral line about one-third the distance from the anterior end and leads directly into the lumen of the muscular pharynx. The mouth varies in length from 5.7 to 7.6 microns and in width from 3.8 to 7.6 microns. The pharynx meets the ventral surface at an angle of 90 degrees and forms an angle of approximately 90 degrees with the intestine. This species has a pharynx which is bulb shaped and about as long as wide, and is composed of one layer of cells each of which has one nucleus at its outer end. The diameter of the pharynx varies from 48.9 to 65.2 microns and its length varies from 48.9 to 65.2 microns.

The intestine is an elongated sac-like structure lying in the longitudinal-median plane between the arms of the horse-shoe-shaped testis which has its open ends towards the caudal end of the animal. Dorsal and ventral to it lie the vitellaria and caudal to it the single slightly lobed ovary. The wall of the intestine is composed of a single layer of columnar cells which joins directly with the pharynx without an esophagus being present. The intestine wall has a minimum thickness of 16.3 and a maximum thickness of 57.0 microns. The lumen varies in thickness from 16.3 to 48.9 microns. The distance of the intestine from the an-

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terior end of the animal varies from 114.1 to 163.0 microns and its distance from the posterior end of the animal from 130.0 to 326.0 microns. In some specimens the intestine is nearer the dorsal than the ventral surface of the worm. In some the anterior end, cephalad to the pharynx, is tipped ventrally and thus the posterior end is nearer the dorsal surface. The pharynx joins the intestine at approximately the posterior end of the first third of the body of the intestine. The length of the intestine varies from 277.1 to 326.0 microns. It is approximately the same distance from the right and left side of the animal and has several lateral pockets where the wall of the intestine pushes out slightly between the vitellaria. These are best seen in frontal sections where six to ten small pockets may be noted.

Female reproductive organs: The ovary is a single slightly lobed organ. It lies in the posterior part of the worm just posterior to the intestine and dorsal to the copulatory organ. The ovary varies in length from 97.8 to 228.2 microns. Its thickness varies from 81.5 to 195.6 microns. The ovary at its greatest width varies from 114.1 to 147.7 microns. The histological structure of the ovary presents an appearance of numerous ova in various stages of development with no apparent matrix or ground substance between them. Each developing ovum has one heavily stained and very prominent nucleolus inside a large nucleus which is usually about one half the diameter of the cell in which it is enclosed.

The oviduct is quite a wide duct coming off of the ovary at its anterior end and leaving the ovary from its ventral side just to the left of the seminal receptacle and about level with the anterior end of the seminal receptacle. It is approximately the same diameter throughout. It swings dorsally and surrounds the ovary passing dorsally, ventrally and laterally to it or passes forward and bends abruptly and then passes posteriorly to the left of the ovary. It joins the vagina anterior to the opening of the duct of the seminal receptacle and slightly posterior to where the vitelline duct is given off from the oviduct. The maximum width of the oviduct varies from 13.3 to 22.8 microns.

The vagina is a large tube coming off the anterior end of the atrium genitale commune dorsal to the cirrus pouch. The anterior end of the vagina begins just posterior to where the vitelline duct joins the oviduct. Its length is 76.0 microns. Its diameter varies from 26.6 to 28.5 microns.

The vitellaria are in two main groups situated in the dorsal and ventral planes of the worm and are connected by branches extending through the worm dorso-ventrally on each side of the intestine and between the arms of the testis. They are fan-shaped, the main branches extending forward and laterally from a point one-third the distance from the posterior end of the vitellaria. There are six main branches in the dorsal

group. These primary branches divide and present a dendritic appearance, each primary branch subdividing two or three times and having rounded lobes at the distal ends of the secondary branches. The ventral group is somewhat more dendritic than the dorsal one. The vitellaria are closer to the posterior end of the worm than the anterior end and about equal distance from the sides. These two fan-shaped vitelline glands are about equally distant from the dorsal and ventral surfaces of the worm.

The vitelline duct enters the oviduct at the lower end of the oviduct just before the oviduct enters the vagina posterior to the ovary. The vitelline duct passes almost straight anteriorly giving off several branches to the vitellaria. It passes forward between the seminal receptacle and the cirrus pouch and terminates by dividing into two main branches just dorsal to the pharynx. The vitelline duct is quite uniform in diameter being slightly larger at its lower end. Its diameter varies from 2.0 to 7.6 microns.

The shell gland surrounds the lower end of the oviduct and vitelline duct at the point where the vitelline duct empties into the oviduct. It consists of slender radiating tubules of varying lengths and is very granular. The length of the shell gland varies from 20.6 to 30.4 microns and its width varies from 22.8 to 38.0 microns.

The seminal receptacle is a globular sac-like organ elliptical in outline and slightly tapering towards its anterior end. It lies just anterior to the beginning of the posterior third of the animal. It is dorsal to the cirrus pouch and lies just to the right of where the oviduct leaves the ovary. It has a heavily walled and ciliated duct which passes posteriorly between the cirrus and the vitelline duct and joins the vagina posterior to the shell gland. The length of the seminal receptacle varies from 64.6 to 72.2 microns and its diameter varies from 34.2 to 38.0 microns.

The uterus is an elongated sac-like structure arising from the ventral side of the cirrus pouch. It lies just ventral to the cirrus pouch in the longitudinal-median plane or slightly to one side of the mid-line. When gravid it contains one egg which greatly distends its walls. The greatest diameter of the uterus is at the upper end where it varies from 19.0 to 81.5 microns.

Only two specimens were found which contained mature eggs with shells. These eggs measured 95.0 and 107.9 microns in length and 72.4 and 81.5 microns in width. The eggs lie in the upper end of the uterus with their polar filaments pointing posteriorly about half way between the lateral margins of the animal in the longitudinal-median line and at the beginning of the posterior half of the animal.

Male reproductive organs: There is a single horseshoe-shaped testis with its open end pointing posteriorly. It lies on each side of the intes-

tine equally distant from the dorsal and ventral surfaces of the worm and approximately equally distant from the right and left sides of the animal with its arms bending slightly towards the median line posteriorly. The testis lies between the fan-shaped vitellaria which are dorsal and ventral to it. The copulatory organ and the common duct of the male and female reproductive systems (atrium genitale commune) leads caudally in the mid-ventral line between the open arms of the testis. In cross section the testis is almost cylindrical and very slightly lobed.

The cirrus pouch is an elliptically elongated organ lying in the longitudinal-median plane nearer the ventral surface of the worm and posterior to the pharynx. The pouch tapers slightly towards its outlet where it turns at an angle of thirty degrees to enter the atrium genitale commune. It enters the atrium genitale commune on its ventral side near the posterior end of the worm. The long axis of the pouch is approximately parallel to the long axis of the body of the worm and varies in length from 163.0 to 304.0 microns. Its width at the anterior end is from 26.6 to 38.0 and at the posterior end from 19.0 to 26.6 microns. The wall of the pouch is muscular, consisting of a thin layer of circular muscle fibers underlaid by a few scattered longitudinal muscle fibers. The prostate gland lies within the muscular sheath at the cephalic end of the cirrus pouch. It is composed of a finely granular matrix and completely surrounds the upper end of the cirrus pouch.

The cirrus is an elongated muscular organ situated in the mid-ventral line nearer the ventral surface at the anterior end of the posterior third of the body and lies in the cirrus pouch. It tapers slightly from the proximal to the distal end and is somewhat curved, usually being curved towards the ventral surface of the animal. It is covered by a thick, smooth, non-cellular cuticula and is unarmed. The cirrus varies in length from 95.0 to 179.0 microns. Its diameter varies from 9.5 at its lower end to 22.8 microns at its upper end. The thickness of the cuticula was 1.9 microns in all specimens. The diameter of the lumen was 3.8 microns.

The vas deferens comes off the posterior end of the cirrus pouch. It opens into the lumen of the cirrus and passes posteriorly through the dorsal wall of the cirrus and cirrus pouch and divides soon after leaving the wall of the cirrus pouch. The two vasa efferentia join the horseshoe-shaped testis in the inner side approximately half way between the anterior and posterior end of the testis. The diameter of the vas deferens is uniform and measures 7.6 microns.

The atrium genitale commune is an expanded tube into which the genital pore empties at the posterior end and the vagina and cirrus pouch at the anterior end. It varies in width from 26.6 to 38.8 and in length from 57.0 to 76.0 microns. The wall of the atrium genitale commune is

composed of low columnar cells which are ciliated. The cilia are long, coarse and far apart. The cilia extend up into the duct of the seminal receptacle its entire length. The length of the cilia vary from 7.6 to 11.4 microns.

The genital pore is at the posterior end of the worm situated equidistant from the right and left sides of the animal and slightly ventral to the longitudinal-median axis. Its diameter varies from 5.7 to 7.6 microns.

The epithelium covering the body varies in thickness from 5.7 to 7.6 microns. The cells of the epithelium are columnar with large nuclei at their basal ends and free from rhabdites. Cilia cover the whole surface of the body.

Host.—*Stichopus* (species unknown).

Location.—Intestine.

Locality.—Bermuda.

On the basis of the above described form the new genus may be defined as follows:

Large pharynx-doliiformis in the first third of the body; genital pore at the posterior end; pharynx originates from the ventral surface of the intestine and forms an angle of 90 degrees with the ventral surface of the animal; one large irregularly lobated ovary; two horn-like branched vitellaria; single horseshoe-shaped testis; large elliptical thick walled seminal vesicle; uterus single; shell gland present; oral sucker absent, intestine a sac with several lateral pockets with the anterior third of the intestine forming a pocket anterior to the pharynx; vas deferens coming off the base of the cirrus pouch and immediately dividing; cirrus pouch present; cirrus long, unarmed and possessing a thick cuticula; prostate gland present; eggs elliptical with one straight uncoiled polar filament as long as the egg; vagina present, dorsal to cirrus pouch; genital atrium commune large, with low columnar cells and long coarse cilia; body of animal entirely covered with cilia.

Acknowledgments. The parasites were collected by Dr. F. D. Barker and the work was conducted under his direction.

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EXPLANATION OF PLATES

All drawings were made with the aid of the Camera lucida from toto, transverse, frontal and sagittal sections of stained and mounted specimens.

Abbreviations

ci., cilia	pr., prostate gland
c. p., cirrus pouch	s. r., seminal receptacle
d., ductus genitale commune	s. g., shell gland
d. s. r., duct of seminal receptacle	s. v. dt., small vitelline duct
e., egg	t., testis
f., filament of egg	u., uterus
g. p., genital pore	v., vagina
i., intestine	v. d., vas deferens
m., mouth	v. dt., vitelline duct
ov., ovary	v. e., vas efferens
ovi., oviduct	v. g., vitelline gland
p., pharynx	

PLATE I

- Fig. 1. Reconstruction of *Macrogynium ovalis* showing digestive system and arrangement of ducts and organs of reproductive system.

PLATE II

- Fig. 2. Frontal section horseshoe-shaped testis.
- Fig. 3. Frontal section through pharynx showing ventral vitellaria and connection of vitelline duct.
- Fig. 4. Median sagittal section through mouth, pharynx and intestine.
- Fig. 5. Toto mount showing ventral vitellaria.
- Fig. 6. Toto mount showing vitellaria on dorsal side.
- Fig. 7. Frontal section through pharynx, vitellaria, egg and ovary.
- Fig. 8. Sagittal section showing vitelline duct and its relation to pharynx and uterus.

PLATE III

- Fig. 9. Histological study of ovary.

- Fig. 10. Sagittal section showing cirrus pouch and ventral to it the uterus and its connection to the ventral side of the cirrus pouch.
- Fig. 11. Sagittal section of pharynx made by cross section of worm.
- Fig. 12. Sagittal section through pharynx, intestine and ovary.
- Fig. 13. Median sagittal section showing connection of cirrus pouch to ductus genitale commune.
- Fig. 14. Trans-section through pharynx showing relation of testis, vitellaria and intestine.
- Fig. 15. Trans-section showing pharynx, mouth, intestinal wall and nuclei.
- Fig. 16. Trans-section through testis in region of ovary.
- Fig. 17. Cross-section of pharynx.
- Fig. 18. Cross-section showing seminal receptacle above, prostate gland in base of cirrus pouch and relation to uterus.

PLATE IV

- Fig. 19. Toto mount ventral view showing egg with polar filament in situ.
- Fig. 20. Cross-section through testis seen at each side with ovary, vitellaria, and intestine.
- Fig. 21. Frontal section through intestine, ovary and testis.
- Fig. 22. Reconstruction of posterior part of animal showing relation of oviduct to vagina, vitelline duct and shell gland, also cirrus, cirrus pouch and uterus in relation to ductus genitale commune.
- Fig. 23. Three serial sagittal sections through testis, vitellaria and dorso-ventral muscles showing in lower diagram.

PLATE I

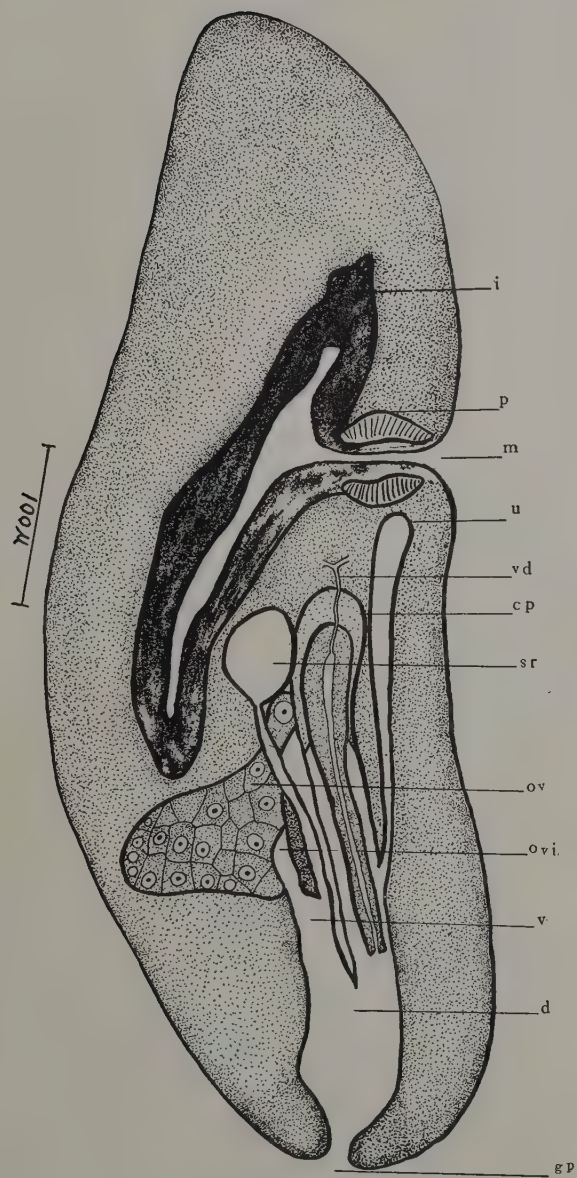


PLATE II

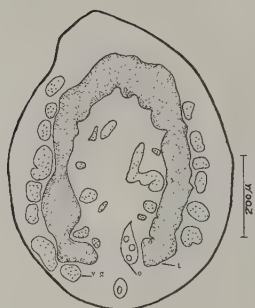


Fig. 2

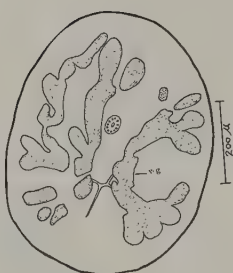


Fig. 3



Fig. 4

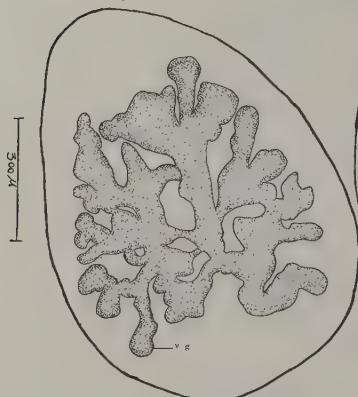


Fig. 5

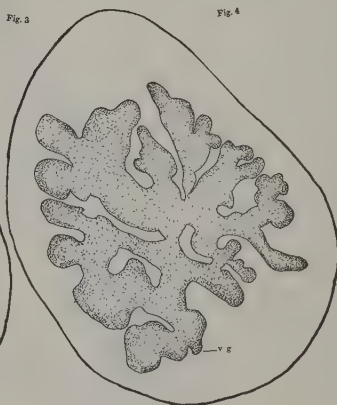


Fig. 6

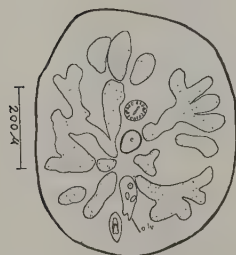


Fig. 7

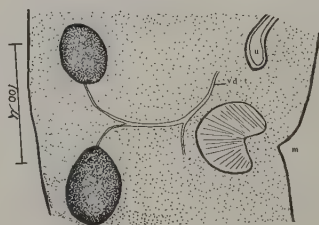
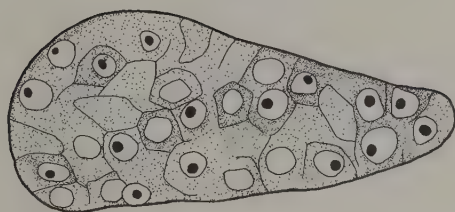


Fig. 8

PLATE III



100 μ

Fig. 9



Fig. 10



Fig. 11

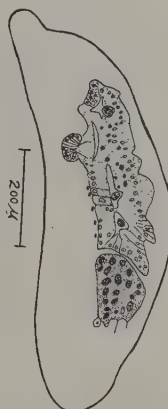


Fig. 12



Fig. 13

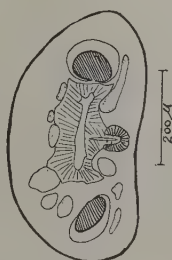


Fig. 14

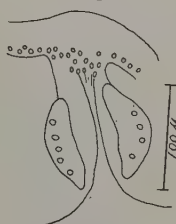


Fig. 15



Fig. 16



Fig. 17

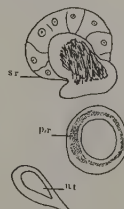


Fig. 18

PLATE IV

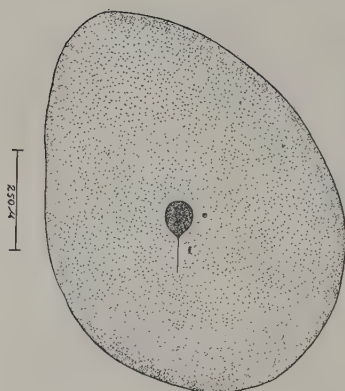


Fig. 19

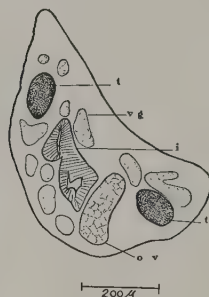


Fig. 20



Fig. 21

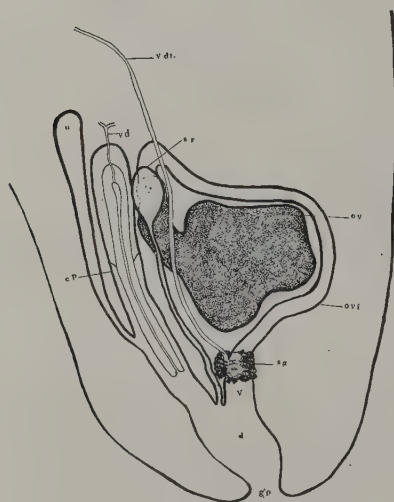


Fig. 22



CROSS-INFECTION EXPERIMENTS ON PARASITE-FREE CHICKS WITH INTESTINAL COCCIDIA FROM THE RABBIT

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In 1931, a student in the Poultry Department of the School of Agriculture at Hampton Institute kept a few pet rabbits in the pen in which he had his flock of leghorns. Some of the rabbits became ill with coccidiosis and a few died. The next week a severe outbreak of coccidiosis swept through the flock causing great loss. Spread of the disease was prevented, but the evidence seemed to indicate that the rabbits had been the cause of the epidemic among the fowls. Since cross-infections with coccidia and host-parasite specificity in the genus *Eimeria* are still moot questions, this seemed a suitable problem for investigation.

MATERIALS AND METHODS

The experimental parasite: After scrapings of the intestinal walls and all the intestinal contents of rabbits newly dead from coccidiosis had been collected in 5 per cent potassium bichromate, the oöcysts were concentrated by a D. C. F. method slightly modified from that suggested by Andrews (1930). They were then allowed to sporulate and were carefully measured and studied so that identification of the species would be possible later if the cross infection proved successful. The sporulated oöcysts were found to contain 4 sporoblasts and a residual body, and the sporocysts always developed 2 sporozoites and a residuum. The micro-pyle was distinct but had no lips. Careful measurements of 353 oöcysts showed a size range as follows: length 25.5 to 37.4 μ , breadth 15.3–23.8 μ , more than 80 per cent being from 29–34 μ long by 19–24 μ broad. After comparing these oöcyst characters with those described by Kessel and Jankiewicz (1931) it was concluded that the fatal coccidiosis of the rabbits studied had been caused mainly by *Eimeria media* (Kessel & Jank, 1931).

The experimental hosts: Twelve parasite-free incubator chicks were used as experimental hosts, as suggested by Hegner (1929a). Measures for isolation and cleanliness were taken so as to prevent accidental infection. For example, the diet consisted of pasteurized "starting mash" and sterilized water. Chicks were fed from 6 to 30 drops of the concentrated suspension of sporulated oöcysts of *Eimeria media*, and their droppings were collected three times daily (Hegner, 1929b). Collected samples were examined at once and also allowed to incubate at 37.5° C. for 6

days, microscopic examinations being made daily during the incubation period.

Experimental procedure and results: Chick No. 2, age 84 hours, before being allowed to take any other food or water, was fed 6 drops of the suspension containing sporulated oöcysts of rabbit coccidia. Within 17 hours the chick began to ail and the feces collected were found to contain mature oöcysts of an average size $32.3\ \mu$ by $22.1\ \mu$. Because of their size and appearance, and because they were mature, these oöcysts were considered to be the fed forms which had passed uninjured through the chick's alimentary canal as Thomson and Robertson (1926, 1926a) showed possible with fish coccidia in man, and as Young (1929) showed in poultry coccidiosis. The illness was believed due to weakness following starvation and thirst and not to infection, for Fantham (1910) estimated the prepatent period in coccidiosis in chicks at 8 days, Johnson (1923, 1927) at 6 days and Young (1929) at about four days. With regular feeding the chick soon began to recover and within 3 days had returned to normal health. Fecal samples examined over 21 days revealed no oöcysts. The chick is still alive.

Chicks 3 and 3a were fed 10 drops; feces collected 3 times daily and examined over a period of 22 days showed no oöcysts.

Chicks 5 and 5a were fed 30 drops; fecal samples collected 3 times daily for 8 days showed no oöcysts on examination. The chicks were killed and their digestive tracts preserved and studied microscopically. No evidence of infection has been found.

Chicks 1 and 1a were fed 10 drops on each of 3 successive days. Chick 1 showed no oöcysts over 17 days; chick 1a was killed on the 6th day after feeding. Macroscopic and microscopic examinations gave negative results.

Chick 4 was fed 30 drops on each of 3 consecutive days; oöcysts believed to have passed passively through were observed for 3 days, but for 14 days after the final feeding no oöcysts were found in the samples collected.

All other chicks were kept as checks under conditions similar to those of the ones experimentally fed. They reacted normally and examination of their droppings collected 3 times daily showed no oöcysts. None of the 12 chicks died, and except for the starved chick No. 2 not even the experimentally fed ones became sick.

The literature on host-parasite specificity has recently been thoroughly reviewed by many writers including Andrews (1927) and Hegner in numerous papers and in his book (1927). Bruce (1920) seems to have been the first to attempt cross-infection experiments with rabbit coccidia, but he did not succeed in infecting cattle. Kessel & Jankiewicz (1931) reported that they failed to infect chicks with any of five species

of coccidia from the rabbit (*Eimeria stiedae*, *E. irredidua*, *E. media*, *E. perforans* and *E. magna*).

The evidence obtained in these experiments seems to confirm the results of Kessel and Jankiewicz (1931), namely, that sporulated oöcysts from the rabbit (*E. media*) did not cause any infection or coccidiosis in baby chicks. It also seems to extend Hegner's view of host-parasite specificity among coccidia to include the *Eimeria* of rabbits with regard to parasite-free baby chicks. In the light of these experiments, therefore, it seems that coccidial epidemics in poultry are not caused by coccidia from the rabbit.

SUMMARY

1. Measurements were made on 353 oöcysts obtained from rabbits that had died from coccidiosis.
2. The species *Eimeria media* described by Kessel & Jankiewicz in 1931 from California rabbits, has been identified in Virginia rabbits.
3. Baby chicks fed large single doses of sporulated oöcysts of rabbit coccidia did not show any evidence of coccidial infection.
4. Baby chicks fed daily with small doses for 3 consecutive days, showed no evidence of coccidial infection.
5. A baby chick fed large doses of rabbit coccidia on 3 successive days showed no evidence of being infected.

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THE MOLLUSCAN INTERMEDIATE HOST OF THE BLOOD
FLUKE, *SCHISTOSOMA JAPONICUM* KATSURADA,
IN THE PHILIPPINES*

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WITH A NOTE ON THE GENUS *BLANFORDIA*

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In the spring of 1932 I received a lot of an amnicolid snail collected near the town of Palo, Leyte, Philippine Islands. As Dr. Marcos A. Tubangui has shown (1932, Philippine Jl. Sci., vol. 49, pp. 295-304, 5 pls.), this snail acts in that locality as the intermediate host of the Oriental blood fluke, *Schistosoma japonicum* Katsurada. After carefully studying the several described Oriental Amnicolidæ, Mr. J. W. Clench and I decided that the snail sent by Dr. Tubangui was *Blanfordia quadrasi* (Möllendorff), originally described as *Prososthenia quadrasi* Möllendorff (1895, Nachrichtsbl. Deutsch. Mal. Ges., vol. 27, p. 138.—Surigao, Mindanao). In order to make certain of the name, some of the snails from Palo were sent to Dr. H. A. Pilsbry, who was able to confirm the identification by comparison with paratypes of *B. quadrasi* received from the *Quadrasi* Collection.

Unaware that Dr. Tubangui had sent specimens to the United States, Dr. Leopoldo A. Faustino submitted part of the same lot, from Palo, to Dr. Bernhard Rensch, of Berlin. Dr. Rensch concluded that the snail represented a new species, which he has recently described as *Oncomelania hydrobiopsis* Rensch (1932, Philippine Jl. Sci., vol. 49, p. 551, fig. 1). From the description and figure there cannot be the slightest doubt that Dr. Rensch dealt with the same snail which we referred to *Blanfordia quadrasi*.

In his paper on the intermediate host of *Schistosoma japonicum*, quoted above, Dr. Tubangui cites our identification, but adds that Dr. Rensch intended to describe it as a new *Oncomelania*. He also gives some good figures of the snail (Pl. 2, figs. 3; pl. 4, fig. 1), comparing them with *Blanfordia formosana* Pilsbry and Hirase (pl. 2, figs. 4), the intermediate host of *S. japonicum* in Formosa.

Since the identity and correct names of the several molluscan hosts of blood flukes are of much practical importance, I have made a new and

* The present paper was sent to the Editor in April, 1933. Meanwhile Dr. Rensch recognized in a brief note (1933, Philippine Jl. Sci., vol. 50, p. 325), that his *Oncomelania hydrobiopsis* is identical with *Prososthenia quadrasi* Möllendorff.

more critical study of the material sent by Dr. Tubangui. A most careful comparison of these snails with a paratype of *Prososthenia quadrasi*, recently received by the Museum of Comparative Zoölogy from Dr. F. Haas, fails to disclose any difference whatsoever that might be regarded as of specific value. I am forced to conclude that *Oncomelania hydrobiopsis* is a synonym of *Blanfordia quadrasi*. In his paper, Dr. Rensch does not compare his new species with *B. quadrasi*; nor does he mention any of the other amnicolid snails from the Philippines, referred to various generic names by Quadras and Möllendorff. Moreover, these snails were also overlooked by L. A. Faustino in his "Summary of Philippine Marine and Fresh-Water Mollusks" (Manila, 1928).

The question of the proper generic name to use for the several small amnicolid snails that may act as intermediate hosts for blood flukes in the Orient, is extremely involved. As pointed out by Dr. H. A. Pilsbry in an Appendix, the late Dr. N. Annandale's revision of this group (1924, Amer. Jl. Hygiene, Monogr. Ser., No. 3, pp. 269-294) only increased the confusion. It is hoped that the following notes, combined with Dr. Pilsbry's remarks, will clear up the matter.

Möllendorff referred his species *quadrasi* to *Prososthenia* Neumayr (1869, Jahrb. K. K. Geol. Reichsanst. Wien, vol. 19, p. 360; type: *P. schwarzzi* Neumayr), a genus based upon amnicolid snails from the Pliocene strata of southeastern Europe. Dr. Pilsbry writes me that he has seen one of Neumayr's species and that he does not feel convinced any of the Oriental snails are congeneric with *Prososthenia*.

Blanfordia A. Adams (1863, Ann. Mag. Nat. Hist., Ser. 3, vol. 12, p. 424; for *Tomichia japonica* A. Adams and *T. bensoni* A. Adams) is the oldest generic name for the Oriental amnicolids of this group.

Oncomelania Gredler (1881, Jahrb. Deutsch. Mal. Ges., vol. 8, p. 120; monotypic for *Oncomelania hupensis* Gredler) differs from *Blanfordia* only in the costate shell, a peculiarity which is probably only of subgeneric importance. If there are other differences, as for instance in the mode of locomotion, these are as yet unknown. The distinction between the two genera based by Annandale upon the structure of the apex of the shell is imaginary, as shown by Dr. Pilsbry in the Appendix. In his recent Manual (1929, Handbuch der Weichthierkunde, p. 150), J. Thiele accepts *Blanfordia* and *Oncomelania* as distinct genera; but, although he includes in *Oncomelania* both costate and smooth forms, he fails to indicate how the smooth species of *Oncomelania* might be separated from *Blanfordia* (apart from the blunt or pointed apex of the shell, a difference which is non-existent). The description he gives of both genera are hardly comparable. This is particularly true of the radula. Of *Blanfordia* he describes the central tooth, while of *Oncomelania* he describes only the laterals and marginals. So far as I could

ascertain, the radulae of *Blanfordia japonica* and *Oncomelania hupensis* show only very minor differences, certainly not of generic value. Rensch's description and figure of the central tooth of *quadrasi* (which he states is typical of *Oncomelania*) agrees exactly with Thiele's account of *Blanfordia*.

Hypsobia Heude (1890, Mém. Hist. Nat. Empire Chinois, vol. 1, pt. 4, p. 173; monotypic for *Hypsobia humida* Heude) was based on a species with a smooth shell and without an external varix to the outer lip. In both these characters it agrees with the subgenus *Vicinia* Pilsbry of *Blanfordia*. It differs, however, from *Blanfordia* and *Oncomelania* in the multidentate marginal teeth of the radula. Quite possibly it is generically distinct, but none of the snails thus far known as hosts of blood flukes can be referred to it.

Hemibia Heude (1889, Jl. de Conchyl., vol. 37, p. 47; 1890, Mém. Hist. Nat. Empire Chinois, vol. 1, pt. 4, p. 167; for several species, including *Oncomelania hupensis* Gredler) was, as Heude states very plainly, merely proposed in lieu of *Oncomelania*, because the latter name seemed inappropriate. Heude included only costulate species. As no genotype appears to have been designated thus far, *Oncomelania hupensis* Gredler is herewith selected as such, thus making it a synonym of *Oncomelania*.

Katayama Robson, (1915, Jan. 30, British Medical Jl., p. 203; monotypic for *Katayama nosophora* Robson) is a synonym of *Blanfordia*, as shown by H. A. Pilsbry in 1915 (The Nautilus, vol. 19, p. 4) and in the subjoined Appendix. It was based upon a smooth-shelled species.

It would seem then that the smooth-shelled Oriental Amnicolidae known to act as intermediate hosts of the blood fluke, *Schistosoma japonicum*, such as *nosophora* Robson, *formosana* Pilsbry and Hirase, and *quadrasi* Möllendorff, should all be placed in the genus *Blanfordia*. The ribbed-shelled species, *hupensis* Gredler, may be left in *Oncomelania*, if one wishes to retain that name in a generic or subgeneric sense.

APPENDIX

Note on the Genus *Blanfordia*

BY H. A. PILSBRY

It is desirable to keep animals frequently mentioned in medical literature as free from nomenclatorial tangles as possible. Some errors in Dr. Nelson Annandale's account of hosts of *Schistosoma* in "Studies on Schistosomiasis japonica" (American Journal of Hygiene, Monographic Series No. 3) call for correction.

Dr. Annandale stated that Adams gave no figures of his *Blanfordia japonica* and *B. bensoni*; but both species were figured by Adams in An-

nals and Magazine of Natural History for 1863, pl. 7, figures 11 and 12. Annandale attempts to separate the form I figured as *B. japonica* (1915, Nautilus, vol. 29, p. 3, fig. 1a) from the species so named by Adams, calling my form "*Blanfordia pilsbryana*," distinguishing it from *B. japonica* A. Adams by the narrower summit and by the "varix less well developed, not extending round the mouth of the shell." The first of these characters depends wholly upon the amount of truncation of the shell, variable with age and individually, as the series before me shows. In the specimen I figured the truncation is small, as in Adams' own figure of *B. japonica*. In Annandale's figure of the same species the truncation is wide—an older individual. It is curious that anyone who has worked with fresh-water snails, which so often lose the early turns by corrosion, would base a specific distinction on that feature. As to the character of the varix, that of my specimens is as well developed throughout as in Annandale's figure of *B. japonica*. It need only be added that Dr. Annandale stated of *B. pilsbryana* "I have not seen this species." I have recently compared my specimens with *B. japonica* received from A. Adams, in the National Museum, and find them identical. "*Blanfordia pilsbryana*" therefore disappears as a synonym of *B. japonica*. Both were from the same place, Sado Island.

The actual type of *Blanfordia bensoni* (A. Adams) should be examined to ascertain whether it is identical with the form I named *hirasei*, as I have stated, or with *B. simplex*, as Annandale thought. *B. bensoni* was described from Yesso (Hokkaido), and from that island I have seen only the large "*hirasei*" form, 8 to 10 mm. long. *B. bensoni simplex* is from the main island and Kyushu, and runs 6 to 7 mm. long. The latter is the size of *B. japonica*, which Adams expressly stated was smaller than *B. bensoni*. It appears therefore that Annandale's synonymy is incorrect, and *B. hirasei* (Pilsbry) is synonymous with *B. bensoni* A. Adams, while *B. simplex* Pilsbry will stand.

Katayama nosophora yoshidai Bartsch (1925, Jour. Wash. Acad. Sci., vol. 15, p. 72) is the form I figured as *Blanfordia nosophora* (1915, Nautilus, vol. 29, p. 3, figs. 2, 2a) from Sakai, a place nearby. It is a little larger than typical *nosophora*, with the varix stronger, but these features seem so variable in several lots examined, including part of the original lot of *nosophora*, that I did not think a distinction could be made. However, if the subspecies be admitted, it occurs as far north as Yamanashi Ken, in the middle of the Main Island, as well as in Kyushu.

From Annandale's remarks (*l.c.*, p. 281) on the protoconchs of *Oncomelania* and *Blanfordia* one can only conclude that he believed the apical views he gave of the latter to show the protoconch (his pl. 36, figs. 13, 16). This is by no means the case. These figures show the calcareous filling secreted at successive stages of growth to close what

would otherwise be an open breach caused by the loss by corrosion of the early turns of the shell.

Annandale correctly stated that "*Katayama*" (*nosophora*) and *Oncomelania* differ by the ribs of the latter, wanting in the former. But he nowhere explains how *K. nosophora* differs from *Blanfordia*. Up to the present time no one has attempted to indicate any distinctions between them. By all characters now known, "*Katayama*" *nosophora* is a species of the genus *Blanfordia*, and should be known as *Blanfordia nosophora* (Robson).

The external difference between the animals of *Oncomelania* and *Pomatiopsis* shown in Annandale's copied figures are largely due to the fact that the *Oncomelania* is at rest, and the *Pomatiopsis* is represented as crawling out of water. When immersed, *Pomatiopsis* moves with the gliding motion of *Amnicola*, and the foot has quite a different appearance.

It is a pity that Annandale's paper, which from its positive tone and handsome illustration will naturally be thought authoritative, turns out on examination to be crowded with misstatements.

In conclusion it may be noted that since *Katayama* is a synonym of *Blanfordia*, it appears that possible *Schistosoma* hosts occur all over Japan, since *Blanfordia* is found as far north as Yesso.

CERCARIA SPHAERULA N. SP. FROM *HELISOMA*
TRIVOLVIS INFECTING CYCLOPS*

LYELL J. THOMAS

A larval trematode of the family Hemiuridae was observed by the writer and a student, Arthur Dean Johnson, while at the University of Michigan Biological Station the summer of 1932. As it is one of the few cystophorous cercaria in this group described from freshwater snails of North America and as notes on the life history were obtained which may shed some light on infestations in marine as well as freshwater forms this preliminary report is given.

Cercaria sphaerula n. sp.

Diagnosis.—Cystophorous cercaria without swimming movements; measured alive, emerged from bulbs, fully extended about 144μ long by 40μ wide at acetabulum. Cuticle with fine transverse rows of spine-like processes from anterior end to about level of pharynx. Integument and parenchyma somewhat opaque. Globoidal oral sucker subterminal opening ventrally, about 18μ in diameter. Acetabulum about 22μ in diameter, evertable, strongly muscular, located in third quarter of body length. No prepharynx. Spheroidal muscular pharynx about 12μ in diameter followed by short esophagus branching into two large caeca which extend laterally for about 50μ almost to heavy walled urinary bladder at posterior end of body. Bulbus about 99μ in diameter completely enveloping cercaria also coiled extensible excretory tube about 150μ in length, adjacent to short fluted caudal appendage which may be extended about 75μ when excretory tube is withdrawn into bulbus. Excretory tube terminated by thick walled bulb and pore preceded by short region studded with refringent granules.

First intermediate host—*Helisoma trivolvis*.

Second intermediate host—*Cyclops vulgaris*.

Location—free in body cavity.

Locality—Grand View, Mullet Lake, Michigan.

OBSERVATIONS AND EXPERIMENTAL INFECTIONS

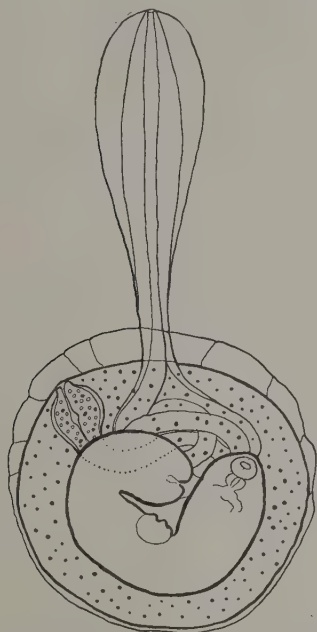
On July 29, 1932, cystophorous cercariae were found in numbers on the bottom of the finger bowl in which *Helisoma trivolvis* had been placed. As they seemed to be about the size of certain Proteocephalid tapeworm eggs which are readily taken by copepods, a mixed culture of Cyclops was placed with them and watched. *Cyclops vulgaris* took the cysts readily so that within the space of two hours some had as many as six metacercariae freely squirming about in the body cavity. When an intra-vitam stain, creysole blue, was used, five cells were counted in the oral sucker, six in the pharynx, and nine in the acetabulum (Text fig. 1, B). Again on July 31, 1932, Cyclops from this same lot were examined and although there seemed to be no great increase in body size of

* Contribution from the University of Michigan Biological Station and the Zoological Laboratory of the University of Illinois.



Text figure 1.—A. *Cercaria sphaerula* within bulbus and excretory appendage everted. B. Cercaria shortly after being eaten by Cyclops. C. Metacercaria from body cavity of copepod about three days later; camera lucida drawing.

the metacercaria within them there was growth, as evidenced by the increased cell number in the pharynx and suckers (Text fig. 1, C). The infected Cyclops were placed with frog tadpoles and minnows, *Notropis*, sp? on the following day and after four days these hosts were teased apart with dissecting needles but no young flukes were found. The experiments are being continued as it is possible that not enough time had elapsed for the development of the metacercaria in the crustacea. Within a few hours after the cercaria had entered the body cavity of the Cyclops, reddish globules were transformed from the host to the intestinal caeca of the larval flukes. The cercaria within the bulbus (Text fig. 2) remained active from five to six days and the snails continued to give them off up to about September 30, 1932.



Text figure 2.—*Cercaria sphaerula* and excretory appendage within bulbus; caudal appendage extended.

DISCUSSION

There are two cystophorous cercaria described from North America, *Cercaria californiensis* Cort and Nichols (1920) from *Physa occidentalis* and *Cercaria projecta* Willey (1930) from *Helisoma antrosa*. Studies on *C. californiensis* were apparently made from specimens removed from the redia and not from normally escaped forms. Although the body of the cercaria is reported as not having the ability to withdraw into the bulbus it may possibly have this feature when mature as is the condition in *C. projecta* and *C. sphaerula*. *C. cystophora* which is described by Wagener (1866) from the European snail (*Planorbis marginatus*) when completely encysted has only the caudal appendage protruding like a handle as in *C. sphaerula*. The main features of most of the cercaria described as cystophorous cercaria by the above workers as well as by Sonsino (1892), Sinitzin (1905, 1907, 1911), Pelseneer (1906), Looss (1907), Yoshida (1917), Faust (1921, 1922, 1924) and Dollfus (1923) include the bulbus, excretory tube, and caudal appendage, although these structures may be variously modified. Willemoes-Suhm (1871), Giesbrecht (1882), Pratt (1898), Cooper (1915), Stafford (1900), Lebour (1923) and Dollfus (1923) have noted metacercaria of

this group free in the body cavity of marine and freshwater copepods or in the act of escaping from them. Other than the observations of Sinitzin (1905) that the cystophorous cercaria of *Halipegus ovicaudatus* develop free in the body cavity of the dragon fly nymph, *Colopteryx virgo*, no experimental life histories have been reported for the group to the knowledge of the writer. Dollfus (1923: 347 and 349) calls attention to the unpublished observations of Maurice Rose (1921, 1922) and to the sketches of Roscoff (1921) which show metacercariae free in the body cavity of the maine copepods *Acartia clausi* Giesb. and *Centropages hamatus* Lillj. He suggests that since *Cercaria calliostomae* agrees with all the characters of the family Hemiuridae and has no cystogenous glands it may likewise have a developmental stage free in the body cavity of a copepod. Now that copepods have been observed in the act of ingesting cystophorous cercariae, the mode of infection in both marine and fresh-water copepoda may prove to be essentially alike and all hemiurids of this family without cystogenous glands may have, after a molluscan host, development free in the body cavities of copepods or aquatic insects that ingest them. Since plankton feeders among fish may acquire tapeworm infections by eating infected copepods and since Thomas (1931, 1931a) has shown that tapeworm infections in frog tadpoles may be acquired through feeding them infected Cyclops it is reasonable to suggest that this may also be another means of infecting frogs with *Halipegus*. *Halipegus* sp? has been taken from frogs in this same locality. There is the possibility too that dragon fly nymphs get their infection of *Halipegus* indirectly by eating infected Cyclops as a large proportion of the food of very young nymphs is microcrustacea and when placed with them in finger bowls they are taken with great avidity.

As there are so many similarities in this group with marine forms it is not unlikely that speculations and observations on the developmental cycle of appendiculate trematodes, such as those attributed to Prof. Karl Möbius by Willemoes-Suhm (1871) and those of Pratt (1898) will need closer scrutiny and experimental evidence.

It has been stated in the literature that the cercariae enter the copepod by eating through the carapace and that they are frequently found in an advanced stage of development with their bodies partly protruding from the copepod. The metacercariae have been observed developing almost to maturity free in the body cavity of a copepod and the host tissues have been fed upon by the larval flukes to such an extent that the host is almost an empty case. They have been seen breaking out of such a container and have been found free in sea water. Numerous plankton feeders among fish and other aquatic animals harbor them. Stafford (1900) and Cooper (1915) have listed many fish hosts with infected microcrustacea and appendiculate flukes free in the stomach. Will

(1844) describes an immature appendiculate fluke from a Ctenophore, *Beroe rufescens* Forskål. Monticelli (1908) found them in Sagitta. The evidence at hand seems to point to the microcrustacea, especially the copepoda, as the second intermediate hosts for the group. The ingestion of copepods by Ctenophores, Medusae, Chaetognaths, dragon fly nymphs and aquatic invertebrates other than the definitive hosts introduces the possibility of accidental or third intermediate hosts which extend the range of the parasite.

SUMMARY

1. *Cercaria sphaerula* n. sp., a cystophorous cercaria from *Helisoma trivolvis* is described.
2. *Cyclops vulgaris* is infected by eating this cystophorous cercaria.
3. Indirect infection of dragon fly nymphs through the eating of infected copepods is suggested.
4. *Cercaria sphaerula* may be a form infecting fish.
5. Because of the similarities of freshwater Hemiuridae to marine forms it is indicated that infections in marine copepods may be possibly due to the direct eating of cystophorous cercaria and by the ingestion of the infected copepods by *Beroe*, *Sagitta*, *Medusae*, and other aquatic invertebrates the range of hosts and parasites is extended.

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FURTHER STUDIES ON THE LIFE CYCLE OF A FROG TAPEWORM *OPHIOTAENIA SAPHENA* OSLER*

LYELL J. THOMAS

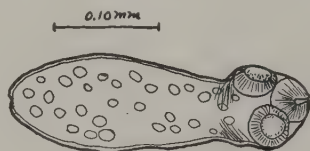
From certain localities near the University of Michigan Biological Station, Cheboygan County, Michigan, frogs have frequently been found harboring tapeworms, one of which, *Ophiotaenia saphena*, was described by Osler (1931). Thomas (1931) discovered experimentally the development of this species in *Cyclops vulgaris* var. *brevispinosus* and in *Mesocyclops obsoletus*, two widely distributed microcrustacea. This paper deals with infections of tadpoles obtained in 1931 by feeding them Cyclops which had been experimentally infected with larval tapeworms of *Ophiotaenia saphena* Osler. It was reported in abstract form December, 1931, for the American Society of Parasitologists but the following details were lacking:

EXPERIMENTS

Laboratory reared copepods, *Cyclops vulgaris* var. *brevispinosus*, were placed in twelve fingerbowls and about one cc. of well water containing innumerable tapeworm eggs from *Ophiotaenia saphena* was added to each bowl. The tapeworms when removed from the frog intestine and placed directly into clear cold water shed enormous numbers of eggs from ripe proglottids. The infection of the copepods was begun on August 11, 1931, and daily examinations showed that there was almost a 100 per cent infection. By August 26th all Cyclops examined showed fully developed proceroids. Tadpoles were obtained August 15 from the mud of Sedge Pool, Douglas Lake, Michigan, which was drying up at the time so that it was literally a wriggling mass of amphibia. Fifty of these tadpoles were teased apart with dissecting needles and the intestinal contents as well as the tissues were examined for the presence of tapeworms but none were found. There were, however, numerous fluke and nematode cysts in some tadpoles. Another fifty tadpoles were saved for the experiment. Twelve of these tadpoles selected at random measured from the tip of the nose to the tip of the tail in millimeters as follows: 15, 22, 15, 16, 16, 20, 18, 20, 16, 18, 16, or an average of 17.3 mm. They were then placed with all the infected Cyclops from the finger bowls in a quart jar August 26, 1931. The other thirty-eight tadpoles were used as controls and placed in another quart jar together with uninfected Cyclops and green algae. As the summer session at the Biological Station was now over the materials were moved by automobile to Madi-

* Contribution from the University of Michigan Biological Station and the Zoological Laboratory of the University of Illinois.

son, Wisconsin. Packed in cold moist sphagnum they withstood the transfer without any casualties and were kept in a cool light basement room until they were taken to Urbana, Illinois, a few days later. The weather in Illinois was extremely hot, 90° F. and when examined September 8, 1931, two of the experimental tadpoles were found floating belly up. Both of them were carefully teased apart under a dissecting microscope but were negative for tapeworms. The controls had in the meantime been distributed among six quart jars and fresh supplies of microcrustacea and green algae were added. It was then decided September 10th to examine the remaining specimens before the heat and school activities interfered. The thirty-eight controls were carefully teased apart one at a time and examined under the dissecting microscope but no tapeworms were found. In each case the digestive tract was removed and examined in a separate dish. On September 11, 1931, the remaining infected tadpoles were examined in the same way and were given host numbers for convenience with the following findings: No. 3—2 larval tapeworms free in the lumen of the intestine near the stomach end; No. 4—1 larval tapeworm free in the lumen of the intestine; No. 5—2 free in the intestine; No. 6—3 larval tapeworms free in the intestine, duodenal region, one drawn by means of the camera lucida, (Text-figure A); No. 7—1 larval tapeworm free in the intestinal lumen; No. 8—3



Text figure A.—Camera lucida drawing of a juvenile tapeworm taken from the intestine of experimental tadpole No. 6.

specimens the same as in No. 6; No. 9—1 juvenile tapeworm; No. 10—no tapeworms found but three larval nematodes encysted in the stomach wall, *Spiroxus* sp?; No. 11—2 larval tapeworms free in the intestine; No. 12—1 larval tapeworm in the intestine. Quantities of algae and an occasional Cyclops were found in the intestinal contents of the control tadpoles. A few Cladocera, Rotifera, and euglenoid forms of Protozoa were observed to have passed through the digestive tracts of the control tadpoles unharmed.

DISCUSSION

Nine tadpoles out of twelve placed with tapeworm infected Cyclops harbored larval tapeworms when examined sixteen days later. Since these twelve tadpoles were not laboratory raised animals, free of para-

sites it is necessary to resort to other data. Innumerable frogs and tadpoles have been taken from this particular pool in the past by the class in helminthology and none have been found to harbor tapeworms. The total number of tadpoles taken at this particular time before the experiment (50) and the controls (38) did not have tapeworms and would seem to justify the writer's conclusion that the infection in the nine tadpoles was induced experimentally. Fortner (1923) did not find frogs infected with tapeworms in the Sedge Point area, where the pool is located, the summers of 1917 and 1919. This survey of the frog parasites of the Douglas Lake region made by him would tend to confirm the findings of the writer that tapeworms have not been found in frogs from the Sedge Pool area. It seems reasonable evidence that since out of one hundred tadpoles examined at the time of this experiment 88 did not harbor tapeworms but 75 per cent of those exposed to infected Cyclops were infected, the nine tadpoles got their tapeworms from eating infected copepods. The average body length of the sixteen larval tapeworms recovered from the nine tadpoles was about $315\ \mu$ as compared with a body length of $162.7\ \mu$ for proceroids from Cyclops. The calcareous bodies had increased to 30 or more in number as compared with 15 to 20 in proceroids. The average diameters of these bodies in both larval stages were about the same. Some growth had evidently taken place during the period the tapeworms were in the intestines of the tadpoles.

During the summer of 1930 and again in the summer of 1931 the writer found larval tapeworms free in the intestines of tadpoles from Fontinalis Run, Burt Lake, Michigan, and mature tapeworms in newly metamorphosed frogs. Both summers larval tapeworms were occasionally taken in adult frogs. Small frogs showing tapeworm eggs in the feces were fed to large *Rana clamitans* and examined two hours later. The tapeworm did not survive the passage through the stomach. This was also true of adult tapeworms poked down the throat of the frog. These experiments were made because, both in the field and in the laboratory, *Rana clamitans* was observed to catch and swallow small frogs. This seems to exclude the possibility of mature tapeworms being transmitted from frog to frog but does not exclude the possibility of larval tapeworms in the intestine of a tadpole or frog being transferred to another frog. More information is needed on this point. Since microcrustacea, Copepoda and Cladocera, were found in abundance in the intestinal debris of tadpoles taken in natural habitats the writer believes that direct infection of frogs or tadpoles in nature may be obtained by their eating infected Cyclops. The finding of mature tapeworms in newly metamorphosed frogs would seem to indicate that juvenile tape-

worms may become mature in the intestine of the same host during the period of metamorphosis.

Hunter and Hunter (1929) have shown that in the life cycle of the bass tapeworm *Proteocephalus ambloplitis* (Leidy) small fish such as fingerling bass, *Aplites salmoides* and *Ambloplitis rupestris*, yellow perch, *Perca flavescens*, the top minnow, *Fundulus diaphanus*, and young pumpkinseed sunfish, *Eupomotis gibbosus* may serve as second intermediate hosts. Meggitt (1914) found that the stickleback, *Gasterosteus aculeatus* Artedi., got its tapeworm, *Proteocephalus filicollis* (= *Ichthyotaenia filicollis*) by eating infected Cyclops without the aid of a second intermediate host. Both methods of infection occur in this family of tapeworms, the Proteocephalidae, known to infect fish.

SUMMARY

1. Frog tadpoles were infected by feeding them *Cyclops vulgaris* containing procercoids of the frog tapeworm, *Ophiotaenia saphena* Osler.

2. The finding of juvenile tapeworms in adult frogs may indicate that the adult has eaten an infected copepod or possibly an infected tadpole.

3. Mature tapeworms in newly metamorphosed frogs suggests that the cestodes undergo either a gradual development during the tadpole stage or a more rapid development as the tadpole begins to metamorphose.

4. The presence of microcrustacea in abundance in the intestines of tadpoles taken from natural habitats affords ample opportunity for natural infections with cestodes.

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STUDIES ON CREPIDOSTOMUM. III. CREPIDOSTOMUM
BREVIVITELLUM N. SP.*

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In the course of Professor Henry B. Ward's investigation of the parasites of fishes of Sebago Lake, Maine, in 1907, eleven eels were examined, of which seven harbored an undetermined species of *Crepidostomum*. This species is represented by about 200 specimens from the eel, *Anguilla rostrata* (Le Sueur), and 5 from the burbot, *Lota maculosa* (Le Sueur). Only one burbot was examined. All of the specimens from the burbot and nearly all of those from the eel are sexually mature. Professor Ward has kindly permitted me to study these specimens, and I have found that they represent a new species, described below as:

Crepidostomum brevivitellum n. sp.

With the characters of the genus (see Hopkins, 1933). Length of mature adults 1.4 to 2.8 mm., width one-ninth to one-fourth of length, sides usually parallel from ventral sucker to posterior testis, body elliptical in cross-section, depth slightly less than width (Figs. 1, 2). Diameter of oral sucker one-eleventh to one-fifth of length (0.24 mm. in specimen 2.0 mm. long); diameter of ventral sucker averaging about three-fourths that of oral sucker. Ventral pair of oral papillae slightly larger than dorsal papillae (length of ventral papillae 120 μ , of dorsal papillae 90 μ in 1.43 mm. specimen); four dorsal papillae equal or dorso-lateral pair slightly larger than median pair. Prepharynx short, muscular. Pharynx ovoidal or ellipsoidal, length one-third to one-half diameter of oral sucker with average two-fifths. Esophagus muscular, dividing at posterior end and then immediately running into intestinal crura, length one to three times that of pharynx. Intestinal crura in dorsal half of body, running from crural fork a short distance in front of ventral sucker nearly to posterior end of body.

Common genital pore median ventral, immediately behind level of crural fork. Cirrus sac very long and slender (length frequently one-fourth that of body), usually sinuous, reaching back over ventral sucker as far as anterior edge of ovary. Seminal vesicle reaching from posterior tip of cirrus sac to its anterior third or fifth, usually somewhat convoluted or crooked, tapering toward anterior end. Prostate glands few and small, mostly in second quarter of sac. Cirrus short, straight or slightly coiled, with rather thin muscular wall. Ovary pear-shaped, near dorsal surface, median or slightly lateral on either side of body, usually some distance behind ventral sucker, often half-way between sucker and anterior testis. Seminal receptacle pear-shaped, immediately behind ovary near dorsal surface. Laurer's canal opening on dorso-lateral surface above intestinal cecum on side farthest from seminal receptacle. Vitelline reservoir close behind seminal receptacle. Oviduct and connecting ducts as in rest of genus (Fig. 4). Uterus tubular, narrow, somewhat convoluted, extending back from oötype to anterior edge or center of anterior testis and then running forward; metraterm, with wall only slightly thicker and more muscular than that of uterus, beginning near posterior edge of ventral sucker and running to common genital pore. Intrauterine eggs 1 to 40 in number, operculate, thin-shelled, ovoidal or ellipsoidal, 50 to 66 μ long by 37 to 46 μ , averaging 60 by 42 μ ; remaining in single-cell stage while in uterus. Vitellaria lateral and ventral to intestinal crura, from posterior edge of ventral sucker to posterior end of body, meeting behind posterior testis

near ventral surface of body. Testes tandem, near ventral surface, spherical to elongate-ellipsoidal; posterior testis usually about twice its diameter from posterior end of body.

Excretory bladder a short undivided sac, seldom reaching forward as far as posterior edge of posterior testis and never (so far as now known) reaching as far as center of posterior testis. Main collecting vessels on each side of body running forward from anterior end of excretory bladder to region between ovary and ventral sucker, where each divides into an anterior and a posterior branch. At least 12 flame cells on each side of body. Excretory pore terminal or slightly dorsal. (Fig. 3.)

Adult in intestine of *Anguilla rostrata* and *Lota maculosa*. Type in collection of Henry B. Ward, slide No. 33-39.

DISCUSSION

C. brevivitellum is distinguished from all other species of Crepidostomum, excepting *C. latum* Pigulewsky, 1931, by the fact that the vitellaria are confined entirely to the post-acetabular region; it is distinguished from *C. latum* by the length of the cirrus sac, which extends far behind the ventral sucker in *C. brevivitellum* but does not reach the posterior edge of the sucker in *C. latum*. The short excretory bladder, reaching only to the posterior edge of the posterior testis or ending behind the testes, is a further distinguishing characteristic of *C. brevivitellum*; so far as is known at present, the bladder in all other species reaches at least to the anterior testis.

I have not been able to determine with any certainty the connections of the finer branches of the excretory system (Fig. 3), but in view of the presence of 12 flame cells on each side it seems probable that the excretory pattern is the same as in *C. cooperi* Hopkins, 1931, *C. cornutum* (Osborn, 1903), and *Megalogonia ictaluri* Surber, 1928, in which the flame cell formula is $2[(2+2+2)+(2+2+2)]$. I worked out the complete flame cell formula and all the connections of the excretory system in these three species from living specimens, while only preserved material is available for study of *C. brevivitellum*; it would undoubtedly be easy to trace out the excretory system of this species from live material, for the tubules and flame cells are exceptionally large. The flame cells have a vibratile cone about $13\ \mu$ long and 4 to $14\ \mu$ wide in sectioned material, becoming wider but not longer in older individuals.

Nothing is known of the life history of *C. brevivitellum*, but its close morphological agreement with the other species of the genus suggests that its life history is probably also similar. The life cycles of *C. farionis* (O. F. Mueller), *C. cooperi* Hopkins, *C. isostomum* Hopkins, *C. cornutum* (Osborn) and probably *C. metoecus* (Braun) are now known in whole or in part, and all show close agreement (see Hopkins, 1934). The cercaria (still unknown in *C. cornutum*) is of the stylet-bearing type with pigmented eyespots (Ophthalmoxiphidiocercaria type of Nöller, 1925), and develops in rediae in finger-nail clams (Sphaeriidae); the

metacercaria encysts in aquatic insect larvae or crustacea. In some of the *C. brevivitellum* adults scattered remnants of the pigmented eyespots can be seen, so this species undoubtedly has an eyespotted cercaria as in the rest of the genus.

The occurrence of *Crepidostomum* in the eel is of some biological interest, since this genus of trematodes is confined to fresh water, while most of the trematodes previously reported from European and American eels are marine forms. Whether the relative scarcity of freshwater trematodes in the eel is due to the fact that the eel is primarily a marine fish to which few freshwater parasites have been able to adapt themselves, or whether it is due to the fact that fewer eels have been examined for parasites in fresh water than in the sea, is not yet known. It may also be worth noting that the burbot, though confined to fresh water, is a member of the only fresh water genus in a marine family. Both of the hosts are bottom feeders.

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PLATE I

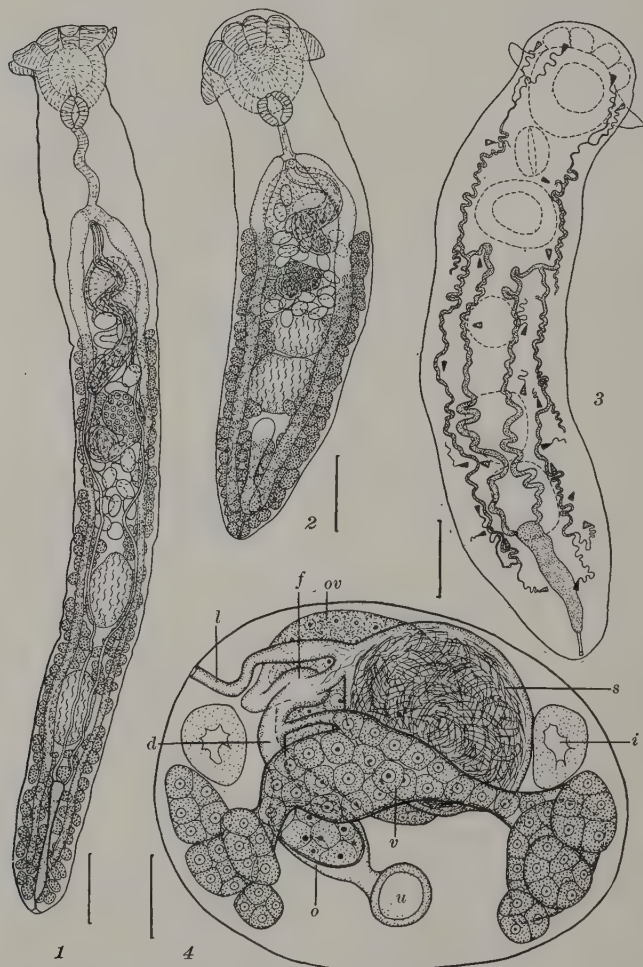


Fig. 1.—*Crepidostomum brevivittellum*, extended specimen, dorsal view of whole mount; scale line represents 0.2 mm.

Fig. 2.—*C. brevivittellum*, contracted specimen, dorsal view of whole mount; scale line represents 0.2 mm.

Fig. 3.—*C. brevivittellum*, excretory system reconstructed from frontal sections; ventral flame cells stippled, dorsal flame cells shown in solid black; scale line represents 0.2 mm.

Fig. 4.—*C. brevivittellum*, female reproductive organs; composite drawing from cross-sections, viewed from posterior end; d—oviduct, f—fertilization chamber, i—intestine, l—Laurer's canal, o—oötype, ov—ovary, s—seminal receptacle, u—uterus, v—vitelline reservoir; scale line represents 0.05 mm.

AN ATTEMPT TO TRANSMIT YELLOW FEVER VIRUS BY
DOG FLEAS (*CTENOCEPHALIDES CANIS* CURT) AND
FLIES (*STOMOXYS CALCITRANS* LINN.)*

MEREDITH HOSKINS

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A number of insects other than mosquitoes have been incriminated as possible carriers of the virus of yellow fever. Philip (1) found that monkey lice (*Pedicinus* sp.) were capable of producing yellow fever when saline emulsions of one hundred or more were injected into a normal rhesus monkey immediately after their removal from infected animals. Davis and Shannon (2) found that in one instance the virus of yellow fever survived for a week in the bodies of *Triatoma megista*. Later Davis (3) found that *Triatoma megista* could produce immunity in rhesus monkeys when injected as late as ten days after an infective blood meal. Injection of triatomas seven days after an infective blood meal resulted in fever and immunity, but injection from three to six days after such a meal produced fever and fatal infection. In one experiment yellow fever virus was transmitted to a normal rhesus monkey by the bites of triatomas after an interrupted feeding on an infected animal. Monteiro (4) reported the experimental transmission of yellow fever by the feces of infected bedbugs, and stated that active virus was eliminated with the feces for as long a period as fifteen days after the infective blood meal. He also claimed that there was no multiplication of the virus within the body of the bedbug. Philip (1) found that the adult, larval, or nymphal forms of bedbugs (*Cimex lectularius*) did not transfer the virus of yellow fever from an infected rhesus monkey to a normal one by direct feeding. Kumm and Frobisher (5) concluded that the virus of yellow fever died off rapidly in the bedbug (*Cimex hemipterus*) and that the disease was not produced by injecting emulsions of bedbugs later than the second day after an infective feeding. Davis (6) found that in certain species of tick the virus of yellow fever may remain virulent for periods as long as twenty-three days. No evidence was obtained that the virus of yellow fever was transmitted by the bites of infected ticks.

The experiments described in this paper were made to determine whether yellow fever might be transmitted by dog fleas (*Ctenocephalides canis* Curt) and the biting stable fly (*Stomoxys calcitrans* Linn.). A

* The studies and observations on which this paper is based were conducted with the support and under the auspices of the International Health Division of The Rockefeller Foundation

dog was used as the source for the fleas. The dog fleas were identified, and all other fleas were discarded. After a period of starvation of from six to eight hours the fleas fed readily when applied to the shaved skin of a monkey. The flies (*Stomoxys calcitrans* Linn.) were captured in stables near the laboratory. They proved to be voracious feeders when applied to the shaved skin of a monkey.

Rhesus monkeys were used in all experiments as a source of virus. These animals had been inoculated with the Asibi (African) strain of yellow fever virus and later died of yellow fever, as determined by microscopic examination of the liver. In most instances the source monkeys had a temperature of 104° F. or more at the time the fleas and flies took their infective blood meals. According to Hudson and Philip (7) the virus is present in the blood stream during the febrile period and also for one day thereafter. In certain experiments a control was used to determine whether virus was present in the blood of the source animal at the time of feeding. Two of the control animals survived after developing fever and later their sera gave a positive protection test in mice, according to the method used by Sawyer and Lloyd (8). In four experiments the source animals were bled after the feeding and the blood was dried. In each instance the dried blood was found to contain virulent virus when injected into normal rhesus monkeys.

The transmission experiments were of two kinds: (1) interrupted feeding experiments in which the fleas and flies were starved for approximately twenty-four hours, then applied to the source monkey and, after feeding for about two minutes, transferred to a normal monkey to finish their feeding; and (2) experiments in which the fleas or flies, after having fed on both an infected and a normal animal, were ground in 10 per cent normal serum and the entire mixture was injected intraperitoneally into a normal monkey.

Table 1 summarizes the results of experiments in which dog fleas were tested as possible carriers of yellow fever virus, and Table 2 shows the results of the experiments made with biting stable flies.

DISCUSSION

The observation that dog fleas (*Ctenocephalides canis* Curt) did not transfer yellow fever virus by interrupted feedings makes the possibility of their being transmitters of yellow fever in nature quite remote. The biting stable fly appears to be able to transmit the virus by interrupted feeding, even after a period of several hours. The fact that these flies will readily feed on a new host after being interrupted makes them potential carriers of some importance. Whether they play any part in nature in the actual dissemination of the virus is not known. The observation that the virus dies out quite rapidly in the bodies of both of

TABLE 1.—Results of experiments using dog fleas (*Ctenocephalides canis Curt*) as possible carriers of yellow fever virus.

Lot	Number of fleas	Source of virus <i>M. rhesus</i> number	Date of feeding	Fever in source animal at time of feeding	Result of infection in source animal	Virus control	Method of testing	Hours after infectious blood meal	Result in test animal	
										Immun- ity
I	13	A 1	7-14-32	Yes	Died 7-28-32 Y.F.*	Blood dried; virus found active	Injection into <i>M. rhesus</i> A 17	72	Survived	None
II		A 2	7-20-32	Yes	Died 7-25-32 Y.F.		Injection into <i>M. rhesus</i> A 18	72	Survived	None
III	18	A 3	7-20-32	Yes	Died 7-24-32 Y.F.					
		A 4	8- 2-32	Yes	Died 8- 6-32 Y.F.		Injection into <i>M. rhesus</i> A 19	24	Survived	None
V	12	A 5	8- 1-32	Yes	Died 8- 4-32 Y.F.					
		A 6	8- 9-32	No	Died 8-11-32 Y.F.		Injection into <i>M. rhesus</i> A 20	24	Survived	None
VII	12	A 7	8-12-32	Moribund	Died 8-12-32 Y.F.					
VIII	13	A 8	8-18-32	Yes	Died 8-22-32 Y.F.	Blood dried; virus found active	Injection into <i>M. rhesus</i> B 1	18	Survived	None
IX	12	A 9	8-23-32	Yes	Died 8-25-32 Y.F.		Injection into <i>M. rhesus</i> B 2	7	Died 8-30-32 Y.F.	
X	14	A 10	8-26-32	Moribund	Died 8-26-32 Y.F.		Finished feeding on <i>M. rhesus</i> B 3	0	Survived	None
XI	14	A 11	8-30-32	Yes	Died 8-31-32 Y.F.	<i>M. rhesus</i> A 14 developed immunity	Finished feeding on <i>M. rhesus</i> B 4	0	Survived	None
XII	12	A 12	9- 9-32	No	Died 9-12-32 Y.F.	<i>M. rhesus</i> A 15 died 9-14-32 Y.F.	Finished feeding on <i>M. rhesus</i> B 5	0	Survived	None
XIII	15	A 13	9-23-32	Yes	Died 9-25-32 Y.F.	<i>M. rhesus</i> A 16 died 10-10-32 Y.F.	Finished feeding on <i>M. rhesus</i> B 6	0	Survived	None

* Y.F.=yellow fever, as determined by microscopic examination of the liver.

TABLE 2.—Results of experiments using biting stable flies (*Stomoxys calcitrans* Linn.) as possible carriers of yellow fever virus

Lot	Number of fleas	Source of virus <i>M. rhesus</i> number	Date of feeding	Fever in source animal at time of feeding	Result of infection in source animal	Virus control	Method of testing	Hours after infectious blood meal	Result in test animal	
										Immun- ity
XI	3	B 7	11-16-31	Yes	Survived	<i>M. rhesus</i> B 13 died 11-25-31 Y.F.	Injection into <i>M. rhesus</i> B 18	72	Survived	None
I	5	A 8	8-18-32	No	Died 8-22-32 Y.F.*	Blood dried; and virus active	Injection into <i>M. rhesus</i> B 19	48	Survived	None
IV	8	B 8	8-31-32	Yes	Died 9-1-32 Y.F.	<i>M. rhesus</i> B 14 died 9-4-32 Y.F.	Injection into <i>M. rhesus</i> B 20	48	Survived	None
VI	10	B 9	9-10-32	Yes	Died 9-13-32 Y.F.	<i>M. rhesus</i> B 15 died 9-14-32 Y.F.	Injection into <i>M. rhesus</i> C 1	42	Died 9-19-32 Y.F.	
II	19	A 9	8-23-32	Yes	Died 8-28-32 Y.F.		Injection into <i>M. rhesus</i> C 2	24	Died 8-31-32 Y.F.	
VIII	17	B 10	10-7-32	Yes	Died 10-8-32 Y.F.	<i>M. rhesus</i> B 16 developed immunity	Finished feeding on <i>M. rhesus</i> C 3	17	Survived	None
IX	26	B 11	10-20-32	Yes	Died 10-23-32	Blood dried; and virus active	Finished feeding on <i>M. rhesus</i> C 4	16	Survived	None
X	6	B 12	1-6-33	Yes	Died 1-8-33 Y.F.	<i>M. rhesus</i> B 17 died 1-12-33 Y.F.	Finished feeding on <i>M. rhesus</i> C 5	16	Survived	None
VII	11	B 9	9-12-32	Moribund	Died 9-13-32 Y.F.		Finished feeding on <i>M. rhesus</i> C 6	6	Died 9-30-32 Y.F.	
V	20	B 9	9-10-32	Yes	Died 9-18-32 Y.F.	<i>M. rhesus</i> B 15 died 9-14-32 Y.F.	Finished feeding on <i>M. rhesus</i> C 7	0	Died 9-16-32 Y.F.	

* Y.F. = yellow fever, as determined by microscopic examination of the liver.

the insects studied decreases the probability of their being carriers of importance.

SUMMARY

Dog fleas (*Ctenocephalides canis* Curt) did not transmit the virus of yellow fever to rhesus monkeys in interrupted feeding experiments, but fleas which had fed seven hours previously on an infected animal caused the death from yellow fever of a normal monkey into which they were injected. The virus was not demonstrable in the bodies of fleas eighteen hours after an infective blood meal.

In an interrupted feeding experiment, biting stable flies (*Stomoxys calcitrans* Linn.) transmitted the virus of yellow fever to a rhesus monkey six hours after the infective blood meal, but not sixteen hours after such a meal. Flies which had fed forty-two hours previously on an infected monkey caused the death from yellow fever of a normal monkey into which they were injected, but injections of flies forty-eight and seventy-two hours after feeding did not produce yellow fever.

I wish to express my appreciation to Dr. Nelson C. Davis for his help and suggestions during the studies here recorded.

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THE LIFE-CYCLE AND BIONOMICS OF *LIPEURUS* *HETEROGRAPHUS* NITZSCH*

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The statement that Mallophaga live but a short time when removed from their hosts is of frequent occurrence in the literature of the group. Few biological studies of this sub-order have been undertaken because of the general acceptance of this opinion. Barber (1921) was the first to show that inter-relations between the host and these parasites may not be as intimate as have been supposed. He succeeded in keeping specimens of *Degeeriella vulgata* Nitzsch. alive in an incubator, with only feathers as food, for 29 days at a temperature of 35° C. During this time eggs were laid, and they hatched. Specimens of *Philopterus subflavescens* (Geoffroy) were similarly kept in an incubator for at least 18 days. Unfortunately Barber's experiments were brought to an abrupt close by a fire in the building in which he was working.

In 1930 the present writer also undertook the study of Mallophaga under controlled laboratory conditions. The mating habits of *Lipeurus heterographus* Nitzsch. were observed and the feeding habits studied experimentally. Data on the life cycle were obtained and are presented. This is believed to be the first report of the elucidation of a Mallophagan life history.

A standard incubator which had a volume of 29,970 cubic centimeters was used. Ventholes 2 cms. in diameter, one in a corner near the bottom, and a similar one in the top, allowed for proper ventilation. Glass dishes exposing a total water surface of about 100 square cms. were placed on the bottom of the incubator to supply the air with moisture.

Feathers were obtained from the neck region of a white fowl, and were cut into two parts. The fluffier basal portion was fastened in the center of a Syracuse watch glass by means of paste applied to the quill. The barbs of the feathers were trimmed so that they did not come in contact with the edges of the watch glass.

A male and a female *Lipeurus heterographus* Nitzsch. of unknown age were placed on the feather in each watch glass. The watch glass was numbered and put in the incubator. The first few pairs died before the most satisfactory temperature was found to be between 33° C. and

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34° C. When an egg was laid, the male and female were placed on a fresh feather in a new dish and the old feather with the egg was labeled; *i.e.*, the watch glass with the first egg of pair No. 12 was labeled 12-1, and later eggs labeled accordingly. Observations were made four times daily, at six hour intervals, and the time at which each egg was laid and the dates on which nymphs molted were recorded.

The following tables (1 and 2) are based on data obtained during the summer of 1930. Other specimens were kept in the incubator until December and bred actively during the entire period. As shown by the tables, the life-cycle from egg to adult, may take from 32-36 days. The occurrence of three nymphal instars is suggestive of a close relationship between the Mallophaga and the Siphunculata which have a similar life-cycle.

TABLE 1.—Data obtained by the study of eggs and young of pair No. 12

Eggs	Dates					Duration			
	Laid	Hatched	1st molt	2nd molt	3rd molt	Incubation	1st instar	2nd instar	3rd instar
12-1	June 23	June 29	Died July 5			6 days			
-2	June 24	July 1	July 8	July 16	July 27	7 days	7 days	8 days	11 days
-3	June 25	July 1	July 9 Died Aug. 7			6 days	8 days		
-4	June 27	July 2	July 9	July 17	July 29	5 days	7 days	8 days	12 days
-5	June 28	July 3	Died July 5			5 days			
-8	July 2	July 9	July 18	Aug. 1	Died Aug. 7	7 days	9 days	14 days	
-9	July 4	July 10	Died July 10			6 days			
-10	July 5	July 10	Died July 22			5 days			
-11	July 5	July 11	Died July 17			6 days			
-12	July 7	July 14	Died July 21			7 days			
-14	July 9	July 16	July 30 Died before molting was complete			7 days	14 days		
-15	July 11	July 16				5 days			

Ten additional eggs were laid and seven hatched. The nymphs were used in other experiments.

From these experimental rearings, four adult lice were obtained. Two were males and two were females. In order to determine whether lice reared in the incubator would mate and produce fertile eggs, the following experiments were carried out. On July 30, No. 16-3, a male was placed on a feather with No. 12-4, a female. On August 2, No. 16-2, a female was placed on a feather with a male, No. 12-2.

The female No. 12-4 laid three eggs, one each on August 6, 7 and 12. The first two eggs hatched on August 13. The female No. 16-2 laid two eggs on August 8; three more were laid; one each on August 9,

TABLE 2.—Data obtained from observations of the eggs and young of pair No. 16

Eggs	Dates					Duration			
	Laid	Hatched	1st molt	2nd molt	3rd molt	Incubation	1st instar	2nd instar	3rd instar
16-2	June 25	July 1	July 7	July 17	July 31	6 days	6 days	10 days	14 days
-3	June 25	July 1	July 8	July 17	July 29	6 days	7 days	9 days	12 days
-4	June 26	July 2	Lost July 7			6 days			
-5	June 27	July 3	Lost July 12			6 days			
-8	June 30	July 7	July 16	Died Aug. 1		7 days	9 days		
-9	July 2	July 8	July 20	Died July 23		6 days	12 days		
-10	July 3	July 10	July 19	Died July 30		7 days	9 days		
-12	July 5	July 11	July 20	Killed July 30		6 days	9 days		
-13	July 7	July 13	July 20	July 30	Killed Aug. 6	6 days	7 days	10 days	

10 and 12. Of the two eggs laid on August 8, one hatched August 13 and the other August 14. Both nymphs were active. No further data were obtained as these experiments were discontinued on August 15.

COPULATION

The males and females of *Lipeurus heterographus* Nitzsch. copulate readily in captivity. They were frequently seen mating when taken from the incubator for examination. For study purposes, however, it was more satisfactory to keep the males and females isolated in separate dishes. After isolation, when males and females were placed together on feathers they would copulate in a very short time and their activities could be observed.

The male when placed in the presence of a female, runs about rather quickly, and the female likewise appears, by her agitated movements, to be aware of the presence of the newcomer. The male moves into close proximity to the female and crawls under her abdomen, so that his dorsal surface is in contact with the ventral surface of the female. Then by a quick movement of his antennae, he seizes the female. The hook-like projections of the third antennal segments are pressed firmly against the sides of the female, usually in the region of the fourth or fifth abdominal segments. This is accomplished by the strong muscles in the head and the enlarged first and second segments of his antennae.¹

Immediately after the male has become attached to the abdomen of the female, he bends his abdomen dorsally and anteriorly until its tip is close to the tip of the female abdomen. The male jerks the tip of his

¹ Mjöberg, 1910, seems to have been the first to suggest the significance of the enlargement and peculiar structure of the male antennae in his observations of *Goniodes falcicornis* Nitzsch. He observed a male grasping the front legs of a female by means of his antennae.

abdomen from side to side and the endomeral plate of the genital armature is extruded. The female, if receptive, usually remains quiet, or may walk about slowly. The tip of the male abdomen is then brought into contact with the tip of the female abdomen and is jerked back and forth laterally, with the tip of the endomeral plate in contact with the region of the female genital opening. This may last for a number of seconds, when the tip of the male abdomen is withdrawn if unsuccessful, and the same tip-to-tip application, accompanied by the lateral vibration of the abdomen, is repeated. The purpose seems to be to spread the genital opening of the female by means of the endomeral plate. The parameres appear laterally against the sides of the endomeral plate, apparently helping to increase its rigidity, and possibly to increase its forward thrust. The female genital opening is located on the ventral side a short distance from the tip, and when opened is immediately entered by the invaginated aedeagus of the male.

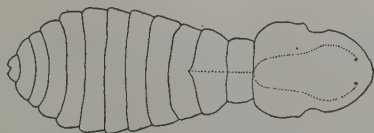


Fig. 1. Outline of the body of a second instar nymph of *Lipaurus heterographus* Nitzsch. The dotted lines indicate where the exoskeleton opens at the time of molting.

MOLTING

The cast skins furnished evidence of the molting process, as the complete process was not observed for any individual. In all the skins studied, the appearance was the same. The dorsum of the head had been split open as indicated by the dotted lines in the outline drawing of the second instar (Fig. 1), and swung forward. The clypeal suture appears to function as the hinge. In the thorax, a dorso-median split appears. This extends forward into the head to connect with the line along which the dorsum split, and may extend posteriorly into the anterior abdominal segments. A few individuals were seen in which the entire body was out of the exuviae except for the anterior tip of the head. This would indicate that the removal of the front of the head is the last step in the molting process. In all the exuviae found, the head portion was bent sharply ventrad and the dorsum swung forward.

FOOD AND FEEDING HABITS

In the life history studies recorded, the lice were given only the fluffier parts of the feather as food. They fed readily, particularly if they were removed from the feather for a time and then replaced. In the process of feeding, a louse generally approached a barb head foremost,

and grasped the barbule with the forelegs. It then cut the barbule from the barb at a rather constant distance. Hanging to the feather with the meso- and metathoracic legs, it forced the barbule forward, base first, between the mandibles with a "hand over hand" movement of the prothoracic legs. Sometimes the head was moved ventrally to assist the legs, but usually the process was carried out by the prothoracic legs alone. On the ventral side of the head, in front of the mandibles, is the labrum which extends to the mandibles, covering them on all sides except the rear. It was observed that the barbule, base first, was fed between the mandibles, and the labrum served as a sack to catch the barbule pieces after they had been cut to the proper length. This sack was repeatedly drawn dorsally and then relaxed, apparently, as it became conveniently filled with the cut-up barbules. This dorsal movement forced the food into the pharynx. Figure 2 shows a normal feather from the neck region of a fowl and a feather on which many lice have fed.



Fig. 2. At the left a feather on which specimens of *Lipeurus heterographus* Nitzsch. have fed extensively and deposited eggs. At the right a feather, from the same region of the fowl, on which lice have not fed.

Experiments with feathers from the Little Green Heron (*Butorides virescens virescens* Linn.) showed that *Lipeurus heterographus* Nitzsch. of the hen will feed on these feathers. The lice were observed eating the barbules, and showed the pigmentation of the feather conspicuously in the intestinal tract. The voided feces likewise showed the pigment, appearing dark in color, whereas the feces were uniformly yellowish white when the lice had been fed on white feathers. The following experi-

ments were tried with various nymphs and adults, using feathers from the Little Green Heron as food.

On July 22nd, three nymphs, just hatched, were placed on a heron feather in the incubator. They were all alive and active eight days later. On the ninth day, one had disappeared, but the other two were active. On the tenth day, one was molting, but died before completely emerging. On the twelfth day, the third had shed its first exuviae but died later in the day.

On September 24th, three first instar nymphs of unknown age were placed on a heron feather. One died five days later during ecdysis, and one died the following day. The third died on the eighth day. The temperature had dropped to an average of 31° C. but other lice on chicken feathers lived under the same conditions.

On September 8th, a male and a female of unknown age were placed on a heron feather in the incubator. Three days later the male was dead. On September 24th the female was dead. Several eggs had been laid, a few hatched, but the nymphs died during ecdysis.

A third instar nymph was placed on a feather in the incubator on September 11th. Twenty days later it shed its exuvia and emerged as an adult female. The next day it appeared pale and no food could be seen in its alimentary tract. It seemed unable to cut off a barbule and was dead a day later.

These lice were in the incubator under the same conditions, except for food, as those under which others of their kind lived. The results of these experiments, therefore, while not conclusive, would indicate that death was due to improper nutrition.

The host specificity of lice has long puzzled students of the Mallophaga. The activities of birds of prey, the quarreling among birds, or any chance contact of bird bodies would make possible transfers between widely related hosts. Similar opportunities for transfer are presented in the activities of mosquitoes and the Hippoboscidae. Notwithstanding these many possible means for migration, collections of lice from birds indicate that, while Mallophaga may be transferred to abnormal hosts, they do not become established.

The above experiments would seem to point toward physiological reasons for species limitations, preventing widespread distribution of Mallophagan species on distantly related hosts. Many experiments to explain their ability to digest and utilize such resistant materials as feathers, are necessary before any substantiated conclusions can be drawn.

BLOOD AS FOOD

The occurrence of blood in the crop of Mallophaga has been considered accidental; due, possibly, to the clot of blood over a wound being

occasionally taken up as food, either alone or with feather. In order more definitely to establish the utilization of blood as a food by these insects, adult individuals of *Lipeurus heterographus* Nitzsch., the same species which in previous experiments had been reared with feather only as food, were placed on a piece of silk fastened in a watch glass. They were given pulverized dried blood from a fowl as their only food, and were placed in the incubator. Two days later a male was found dead but one of the females had blood in her crop, which was conspicuous since the dark pigment of the blood showed through the body wall. All were dead at the end of the third day. On the same day that the above lice were placed in the incubator with blood alone as food, another group of four adults were placed on a white feather to which had been added some pulverized fowl blood. They were all alive three days later: one which had blood in the crop was killed for an intestinal smear. Two days later, one of the three which had eaten blood was placed on a piece of silk with blood alone. Four days later she was dead. The adults, that had blood and feathers available, ate both, and many eggs were laid. As the young hatched and fed, the crops were almost continuously filled with pigmented material which they could obtain only by eating the dried blood.

Two first instar nymphs, hatched from eggs laid by females fed on feathers and blood, were placed on silk with only dried blood for food. They were both dead at the end of the day. Two more first instar lice which had eaten blood were placed on silk with dried blood alone. On the next day they were active and had blood in their crops. One died the second day and the other the day following. Both had blood in their crops when they died. The maximum time for which first instar nymphs could be kept alive on dried blood alone was three days. This was likewise true of the adults. The adults and young were alive and active on the feather with blood for eighteen days, had been seen with blood in their crops nearly every day and were alive and active when the experiments were brought to an end.

These results show that this species relies on feathers for its essential food supply but supplements this with blood when it is obtainable. That this is not true of all biting lice is suggested by the writer's failure to rear *Menopon gallinae* Linn. and *Menopon stramineum* Nitzsch. or even to induce them to lay eggs, although they were kept under the same conditions in which *Lipeurus heterographus* thrived. The writer's, "Observations of a Mallophagan, *Menopon stramineum* Nitzsch., actively feeding on the blood of its host" (Science, 1933), indicates quite different food habits in the last mentioned species.

SUMMARY

Lipeurus heterographus Nitzsch. has been reared in an incubator under controlled laboratory conditions, with feathers as the only food. The incubation period of the eggs observed varied between 5 and 7 days. The first instar has a duration of 6 to 14 days; the second instar, 8 to 14 days, and the third instar, 11 to 14 days. The adult appears following the third molt. Adults reared in captivity were mated, and laid fertile eggs.

Molting and copulation are described in detail. Feeding experiments utilizing feathers, feathers and dried blood, blood alone, and feathers from a host widely separated from the normal host of this Mallophagan parasite, are described and discussed.

LITERATURE CITED

- Barber, B. A. 1921.—Notes on the life history and habits of Mallophaga. Papers Mich. Acad. Sci. Arts and Letters, Ann Arbor, 1921, p. 391-395.
Mjöberg, Eric. 1910.—Studien über Mallophagen und Anopluren. Arkiv. f. Zool., 6: 1-296.

RESEARCH NOTES

TICKS FROM TAPIRS OF PANAMA

This note records the ticks collected from three specimens of *Tapirella beardi* (Gill) in Panama. Tapir No. 1 was a young female (weight 396 pounds) killed near Summit, Canal Zone. The ticks were collected a few hours after death, before many ticks had left the carcass. The other two animals were very young specimens, not yet weaned, captured near Aguas Buenas, Republic of Panama. No. 2 appeared to be 4 to 6 weeks old, No. 3, 8 to 10 weeks old. The ticks were collected from these two small tapirs during life.

On the young animals the ticks were generally distributed, but on the adult tapir they were much more numerous on the ventral surface, especially on the breast between the fore legs and on the upper inner parts of the hind legs.

The species of ticks collected were as follows:

Amblyomma tapirellum Dunn (Parasit., 25: 353-355, 1933). No. 1, 169 adults; No. 2, 88 adults; No. 3, 62 adults. The most abundant species present.

Amblyomma oblongoguttatum Koch. No. 1, 45 adults; No. 2, 23 adults; No. 3, 16 adults. Also abundant, attaching especially about the head and neck.

Amblyomma coelebs Neumann. No. 1, 30 adults; was also present on the two smaller animals.

Amblyomma cajennense (Fabricius). No. 1, 7 adults; No. 2, 2 adults; No. 3, 1 adult. Although rare on the tapirs this species is very abundant in Panama on a wide variety of hosts.

Amblyomma ovale Koch. No. 1, 5 adults; No. 2, 5 adults, No. 3, 2 adults.

Amblyomma humerale Koch. No. 2, 1 adult. This tick is normally found on the tortoise.

Haemaphysalis kochi Aragao. No. 1, 37 specimens; No. 2, 3 adults; No. 3, 2 adults. This species is common on deer in Panama, but I have seen no previous record from tapirs.

In addition to the adult specimens recorded above, larval and nymphal ticks were extremely abundant on all three tapirs.

Since these tapirs are commonly found in low swampy areas and frequently enter the water to feed on aquatic vegetation or as a place of refuge, it seems rather remarkable to find their tick infestation so heavy, both in individuals and species.

Goldman (Smiths. Misc. Coll., 69: Publ. 2498, p. 83, 1920) has also noted the heavy tick infestation of this species in Panama, so that it is probably of common occurrence.—LAWRENCE H. DUNN, *Gorgas Memorial Laboratory, Panama.*

UNIONICOLA ACULEATA (KOENIKE) IN SNAILS IN NORTH CAROLINA

During August, 1933, ten snails, *Campeloma decisum* (Say), were examined from Nancy Rhodes Pond near Durham, N. C. Nine of these contained 2 to 9 mites, besides encysted young in the gills. Parasitic mites have been reported as occurring in snails in South America (Wolcott, R. H.: The water-mites, Hydra-carina; Ward & Whipple's Freshwater Biology, 851-875. 1918), but have not been recorded from hosts other than clams in North America. Mites were found in snails at the same locality during the summer of 1934.—E. R. CHANDLER, *Duke University.*

PIROPLASMA CANIS IN FLORIDA

Through the courtesy of Dr. T. J. Mahaffy, of Jacksonville, I have recently had the opportunity of studying the blood of a dog which harbored *Dirofilaria*

immitis. In addition to the microfilariae, I found intra- and extra-corpuseular organisms that were strange to me. These have been identified by Dr. Bruce Mayne, of the United States Public Health Service, as *Piroplasma canis*, a tick-borne Haemosporidian said to cause malignant jaundice in dogs. This observation is of interest in view of the remarkable increase in the prevalence of dog-ticks in Florida. The literature available to me does not contain any previous record of *P. canis* from this region.

Two points are of especial interest. (1) The piroplasms disappeared from the blood, as did the microfilariae, after the intravenous injection of a proprietary organic antimony preparation. (2) The piroplasms showed an especial predilection for reticulocytes, comparable to that which I have recently shown to characterize the human malaria parasites, *Plasmodium vivax* and *P. falciparum*.—PAUL EATON, State Board of Health, Jacksonville, Florida.

INGESTION OF EGGS AND LARVAE OF *STRONGYLOIDES* BY INTESTINAL CILIATES

Stools from chimpanzees maintained at the Johns Hopkins Medical School contained living specimens of the large ciliates *Troglodytella abressarti* and *Balantidium coli*, and eggs and larvae of the nematode worm, *Strongyloides fülleborni*. On nine different occasions, a larva of *S. fülleborni* was noted inside of a trophozoite of *T. abressarti* and an egg of the worm was observed within the ciliate on four different occasions. A larva was once observed to penetrate a *Troglodytella*, with the result that the protozoon died immediately. During one observation, under a sealed coverslip on a slide, a *Troglodytella* containing a strongyloid larva was watched for more than ten hours; at the end of this time, the larva had ceased to move but the ciliate was still alive. Eggs of *Strongyloides* were twice noted within trophozoites of *Balantidium*. In one case, the egg hatched after the protozoon had died and commenced to disintegrate. The larva then forced its way out. The eggs of *Strongyloides* are about two-thirds the size of a *Balantidium* and one-third that of a *Troglodytella*, so it is evident that these ciliates must have extremely elastic cytostomes.—W. W. SWEZEY AND F. O. ATCHLEY, Johns Hopkins University, School of Hygiene and Public Health.

RHABDOMETRA ODIOSA (LEIDY, 1887) JONES, 1929, A CESTODE PARASITE OF *PEDIOECTES PHASIANELLUS* IN QUEBEC

Following the studies upon the abnormal migration of northern Sharp-tailed Grouse in Quebec (1932-33), Dr. D. A. Dery, Ornithologist to the Quebec Zoological Gardens, submitted to this Institute a number of these birds that died after a short period of captivity. In some cases the cause of death was undoubtedly a form of Blastomycosis, but other birds were heavily infested with cestodes and showed no signs of fungus disease. After a careful study of twenty well preserved specimens, the cestodes concerned were identified as *Rhabdometra odiosa* (Leidy, 1887) Jones, 1929. However, in these, the author noted a great range in size in several organs that appears to be worthy of special note.

Morphology: General characteristics typical of genus. Strobila 2.7-4.0 cm. long by 1.4-2.0 mm. wide. Diameter of scolex 0.380-0.470 mm.; diameter of suckers 0.179-0.256 mm. Mature segments much broader than long; gravid segments one and one half times as long as broad. Genital pore anterior to middle of margin of proglottid; genital canal dorsal to longitudinal nerve. Cirrus pouch in mature proglottids 0.258-0.406 mm. long by 0.067-0.081 mm. wide. Testes vary greatly, from 15 to 43 in number and from 26 μ by 20 μ to 60 μ by 46 μ in size; many are globular and an average of 40 μ in diameter. Testes numbering 43 in one proglottid average 28 μ in diameter; with fewer present the size tends to be greater: those approximating the ovary are the smallest. Parauterine organ large and twisted in gravid segments. Other female reproductive organs as described by M. F. Jones (*Proc. U. S. N. M.*, lxxv, Art. 20, 1929). Onchospheres globular to sub-globular, 28.4 μ in diameter to 30 μ by 28 μ .

Notwithstanding the fact that the size, shape and number of the testes do not agree with *R. odiosa* as described by Jones, and that other features show smaller differences, the author does not feel justified in erecting a new species for these specimens. The variations noted in the morphology of the testes are probably due to methods of fixation, as may be the size of the strobila when the elongated segments as noted by Jones are considered. These Canadian specimens also agree with the available description of *Rhabdometra tomica* Kholodkovsky, 1906, except in the length of the strobila and the size of the onchospheres. As pointed out by Jones, even the differences noted between Kholodkovsky's specimens and those of Leidy were of doubtful value, whereas my Canadian specimens cover these differences except as regards the two aforementioned features. It is, therefore, proposed that *R. tomica* Kholodkovsky, 1906, be regarded as a synonym of *R. odiosa* (Leidy, 1887) Jones, 1929, and that the specific diagnosis of the latter be amended to include the characters of the specimens here described. This proposal is made in spite of the differences in geographical distribution and hosts, which do not appear to be valid reasons for retaining *R. tomica* as a separate species in face of these morphological data.—W. E. SWALES, *McGill University, at Macdonald College, Que.*

AN ANOMALOUS *ASCARIS LUMBRICOIDES* FROM SWINE

While experimenting in vitro with large numbers of *Ascaris lumbricoides* of swine I encountered a mature female worm about nine inches long which possessed a forked "head." When fixed with hot cupric phenol solution the head was offset to the right. On the right side on the level of this offset, which was about 2 mm. from the anterior end, was a secondary "head" a little over 1 mm. long. Both "heads" were examined under a dissecting microscope and found to possess three lips each. However, the lips on the accessory "head" were relatively small and did not cover the mouth opening. It was not possible to distinguish between the dorsal lips and the two lateral lips by such means. The specimen was sectioned, and it was found that the lumen of the esophagus of the accessory "head" communicated with the lumen of the main esophagus, but as the sections did not come out well no further details could be made out. The accompanying photomicrograph was taken from the preserved specimen. The



black spots are a tar which had collected on the "head" during certain in vitro experiments which were performed on this worm before the supernumerary "head" was noted.

Anomalous structures among the nemaodes are relatively rare when compared to the flatworms. However, several female *Ascaris* with abnormal genital organs have been reported (Harms, 1906. Zool. Anz. 30: 486-488 and Spindler (1931. J. Parasitol. 18: 45) has described *Oesophagostomum* females with abnormally short and blunt tails.—PAUL D. HARWOOD.

ABNORMAL PARABASAL SPINES IN THE MALE HYPOPYGIUM OF *ANOPHELES PSEUDOPUNCTIPENNIS*

Dr. Martini (Proc. Ent. Soc. Wash., 35: 64, 1933) has noted the duplication of one of the parabasal spines in a hypopygium of *Anopheles maculipennis* and mentions having seen the same abnormality in hypopygia of *A. pseudopunctipennis*. It seems worth while to record the fact that abnormalities of this sort occur much more frequently in *A. pseudopunctipennis* than in most species of *Anopheles*, and that they seem to occur more frequently in certain portions of its range than in others.

The table below summarizes the numbers of parabasal spines present in my material from several different localities. In specimens from Maracay, Venezuela, about 25 per cent of 123 hypopygia show duplication of one or more parabasal spines. In most of the abnormal specimens, only a single spine is duplicated, as in the cases mentioned by Martini, but in several specimens both of the side pieces have three parabasal spines, and in one specimen one of the side pieces has three spines and the other has four. In 129 hypopygia from the state of Vera Cruz, Mexico, on the other hand, only about 12 per cent are abnormal. About the same rate is found in 16 hypopygia from Panama, and in 46 hypopygia from the island of Grenada the proportion of abnormal is even less (about 9 per cent).

In the Mexican series there is one specimen in which the parabasal spines are reduced, only one being present on each side piece instead of the usual two.—FRANCIS M. ROOT, *School of Hygiene and Public Health, The John Hopkins University, Baltimore.*

Anopheles pseudopunctipennis. Parabasal spines of male hypopygium

Locality	Reduced	Normal	Increased		
	1-1	2-2	2-3	3-3	3-4
Maracay, Venezuela		92	21	9	1
Vera Cruz, Mexico	1	114	10	4	
Grenada, British West Indies		42	4		
Panama Canal Zone		14	2		

A NEW NYCTERIBIID FROM FLORIDA

Among some bat parasites collected by Dr. E. T. Boardman from *Myotis austroriparius* at Gainesville, Florida, were found two females and a male of a *Basilia* which appeared to be undescribed. Dr. G. F. Ferris, to whom figures and a description were sent, states that this is a new species of which he has recently received specimens from the same host and locality.

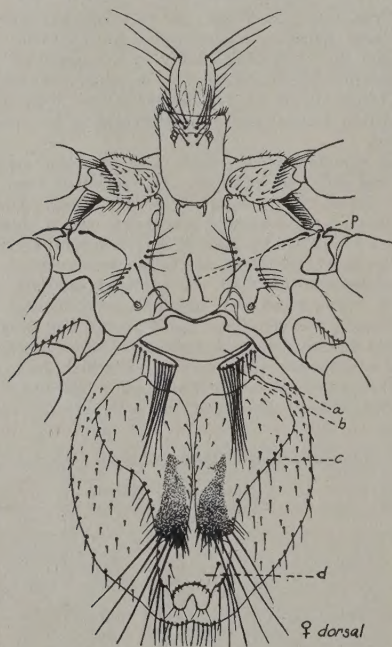
Basilia boardmani new species.

Diagnosis.—This species differs from those previously described in the form of the abdominal tergites of the female and especially by having the mesonotum of the female prolonged posteriorly as an upright, finger-like process (this character was pointed out by Dr. Ferris, who says, "no other Nycteribiid that I have ever seen has anything like it").

Description.—Female. Head as usual in the genus. Eyes two-faceted. Thorax as usual in the genus, except that the mesonotum is prolonged posteriorly in the form of an upright, finger-like process (p). Tarsal claws deeply cleft.

Dorsum of abdomen with first tergite (a) small and oval. Second tergite

(b) divided, the halves widely separated, each consisting of a narrow, strongly chitinized strip bearing five or six very long setae and about four short ones. Third tergite (c) very large, heart-shaped, divided in the median line by a narrow suture. The surface bears a number of small setae, the lateral margins longer setae, and the heavily chitinized, truncate, posterior lobes bear from three to five very long setae and numerous short, spine-like setae. Fourth tergite (d) indefinite anteriorly but strongly chitinized along the postero-lateral margins, which bear four or five long and several short setae.



Basilia boardmani n. sp. Dorsal view of body of female.

Ventral surface of abdomen with the basal sternite covering about one-third of the length of the abdomen, having numerous short setae on its surface and a ctenidium of about sixty spines along the posterior margin. The connexivum caudad of the basal sternite thickly set with short setae and with three transverse rows of longer setae, the first row of six long and widely spaced setae, the second of numerous shorter setae, and the third row, at posterior margin of connexivum, of many short, stout setae. Caudad of the connexivum are a pair of broadly semicircular plates, almost meeting in the median line, with a row of fairly long setae along their curved posterior margins. Caudad of these, a broad plate with many short surface setae and a marginal row of rather long and slender setae posteriorly. Caudad of this is the terminal segment.

Male.—Essentially identical with the male of *Basilia antrozoi* (Townsend), except that a rudiment of the finger-like process can be detected at the posterior end of the mesonotum.

The type slides will be deposited in the U. S. National Museum.—L. E. ROZEBOOM, Division of Medical Entomology, School of Hygiene and Public Health, The Johns Hopkins University.

AMERICAN SOCIETY OF PARASITOLOGISTS, ANNOUNCEMENTS

Preliminary announcement of the 10th annual meeting of the American Society of Parasitologists at Pittsburgh, Penn., Thursday, Friday and Saturday, December 27-29, 1934.

Pursuant to action of the Society taken at the last annual meeting in Boston, the A. S. P. will hold a three-day program in conjunction with the meeting of the A. A. A. S. at Pittsburgh. The headquarters hotel and the location for the regular sessions have not been assigned.

The program will be arranged in accordance with the outlines that have been developed in recent years. The demonstration tea, which has proved so successful in the past, will be continued as an especial feature of the Pittsburgh meeting. The presentation of scientific results by demonstrations and charts is a much more satisfactory method than a formal and limited verbal report. This method permits the presentation of many papers simultaneously, allows each member unrestricted time for the presentation of his material, and encourages the discussion of particular features in a more intimate and detailed manner than is possible after the formal reading of papers. Furthermore, members are not required to listen to reports in which they are not interested. The demonstration tea is both a scientific program and a social function, a place where members can meet informally for the discussion of research or for friendly banter. In every possible instance members are urged to present papers by demonstration. Abstracts of such papers should be prepared in the usual form.

The December number of the JOURNAL OF PARASITOLOGY will contain the program and abstracts of the annual meeting, the roll of members of the Society, and the index of volume 20. This will place unusual pressure on the editorial committee and since it is important that the December number be in the hands of all members before they leave for the meeting, special precautions will be taken to avoid delay. The editorial committee has requested that the final copy of the program be in the Baltimore office on the first of November. Accordingly, all abstracts must be in the office of the secretary on or before October 25th, 1934.

In conformity with the present tendency to curtail expense, the Society will not hold an annual dinner. Reverting to an earlier custom, revived last year, there will be a luncheon, followed by the annual business meeting.

HORACE W. STUNKARD, *Secretary.*

JOURNAL OF PARASITOLOGY

ANNOUNCEMENT OF CHANGE IN SUBSCRIPTION PRICE AND TIME OF PUBLICATION

The Editorial Committee takes this opportunity to announce for the Editorial Board of the JOURNAL OF PARASITOLOGY and the Council of the American Society of Parasitologists the following changes in the JOURNAL OF PARASITOLOGY beginning with volume 21.

1. The December, 1934, number of the JOURNAL will be number 6 of volume 20 and will be the final number in that volume. It will contain the program, abstracts, and announcements for the 10th annual meeting of the American Society of Parasitologists.

2. Volume 21 of the JOURNAL will coincide with the calendar year, being published in 1935.

3. Each volume of the JOURNAL will consist of six numbers which will be published bimonthly in February, April, June, August, October, and December.

4. The subscription price beginning with volume 21 will be raised to four dollars (\$4.00) (Foreign, \$4.25) per volume.

W. W. CORT, *Chairman,*
Editorial Committee.